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PROGRESS OF GERMINATION OF SEED OF DIGITARIA AS INFLUENCED BY GERMINATION TEMPERATURE AND OTHER FACTORS [1]

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INTRODUCTION

That different kinds of seeds respond differently to germination temperature has long been recognized. The work of Crocker (*1, 2*)[2] indicated that these responses are not only associated with the behavior of the cells of the embryo, but are complicated by the coat restrictions. It may be assumed that some fundamental principles are involved in these varied responses of different seeds. A knowledge of the detailed behavior of many kinds of seeds that have different adaptations is needed before valid generalizations can be made. A study of the well-recognized special germination requirements of the seeds of many weeds should furnish information of value in solving the general problem of seed germination. Also, a knowledge of the factors influencing the germination of seeds of specific weeds should aid in their control. The seeds of the two common crabgrasses *Digitaria ischaemum* (Schreb.) Muhl. and *D. sanguinalis* (L.) Scop. were selected for study.[3]

Only incidental references in the literature to the germination requirements of *Digitaria* have been found. Weber and Kirchner (*15, p. 282*) mention that germination of the seed of *D. sanguinalis* is slow and that light is required. Heating the seed at 35° C. had a beneficial effect in hastening germination. Edwards (*4*) has reviewed the literature on the relations of temperature to germination of various seeds, dealing mostly with the response to temperatures maintained at a constant level; Harrington (*8*) has referred to the early work on the effect of daily alternation of temperatures on seed germination; and Morinaga (*13*) and many others have presented work on this phase of temperature relations. The beneficial effect of prechilling, i. e., holding the moist seeds at a temperature slightly above freezing before subjecting them to ordinary temperatures for germination, was first described by Davis and Rose (*3*). The method has been used by many workers for various types of retarded germination of seeds. The changed response of many seeds with age after harvest has been noted by various writers. Harrington (*7*) has reviewed the early literature on the afterripening of cereals. Kearns and Toole (*10*) have recently followed the change with age of the response of fescue seed to alternations of temperature.

[1] Received for publication December 27, 1940. This investigation was conducted in the former Division of Seed Investigations, Bureau of Plant Industry.

[2] Italic numbers in parentheses refer to Literature Cited, p. 90.

[3] A preliminary report of some portions of this study was given before the Botanical Society of America in December 1938.

MATERIAL AND METHODS

Seed collections were made in 1935, 1936, 1937, and 1938 from growing *Digitaria* plants by lightly rubbing the mature inflorescences to remove only the mature seeds. The seed was cleaned to remove chaff and undeveloped fruits and stored in paper bags in the laboratory.

The "seed" as used in these germination studies consisted of the mature fruit or caryopsis enclosed by the close-fitting cartilaginous lemma and palea and the persistent second glume and sterile lemma. Because of the uneven ripening of the seed of these grasses and the tendency of the seed to fall from the plant as soon as it was fully mature, there undoubtedly was a considerable and variable degree of immaturity in the material used.

All germination tests were carried out on moistened paper toweling in Petri dishes. Alternation of temperature was obtained by transferring the dishes from one controlled-temperature chamber to the other. The first-mentioned temperature of an alternation was maintained for approximately 18 hours and the second for 6 hours each day. Seedlings that had developed a definite root and plumule were counted and removed every 7 days, and results usually are reported for 28-day periods.

The temperature of germination chambers above room temperature was maintained by thermostatically controlled electric lamps within the chambers. No attempt was made to exclude light except in special tests in which the Petri dishes were enclosed in tin boxes. Definite exposure to light was made only at the condition designated "20° to 30° with light," in which, for the approximately 6-hour period at 30° C., the germination tests were carried on in a glass-enclosed chamber in a north window.

EXPERIMENTAL RESULTS

Preliminary tests in 1935 at various temperatures indicated that freshly harvested seed of both *Digitaria ischaemum* and *D. sanguinalis* would remain for several weeks with practically no germination under conditions ordinarily favorable for germination, but that when germination finally started it progressed steadily although slowly until practically all (95–98 percent) the viable seed had germinated, which required several months. The final germination was about the same when the seed was maintained at different daily alternations of temperature, but the course of germination was very different. Prechilling the seed shortened the time before germination started and hastened the rate of germination. Tests started after the seed had been stored dry for 2 months in the laboratory showed a marked change in the course of germination.

These preliminary results led to a more detailed study (1) of the comparative response of the two species to a series of daily alternations of temperature, (2) of the change of this response as the seed aged, and (3) of the response of the seed after prechilling for various periods.

EFFECT OF SEVERAL TEMPERATURE ALTERNATIONS ON GERMINATION OF SEED OF DIGITARIA ISCHAEMUM

Freshly Harvested Seed

Germination tests were started within 2 days after harvesting in the fall of 1936, 1937, and 1938, and four alternations of temperature

(20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°) were used. The results of these tests, as presented in table 1, show considerable variation in the germination of the different samples at a given temperature. The relative progress of each sample is very similar for the several temperatures, however, and it is believed that the means for the four samples (fig. 1) represent with reasonable accuracy the relative response to these temperature conditions of the freshly harvested seed of *Digitaria ischaemum*.

The general forms of the curves for germination at alternations 20° to 40° C., 20° to 35°, and 20° to 30° are similar. A more or less lengthy initial period without germination or with only slight germination was followed by a period of comparatively rapid germination, after which the rate became much slower and the germination of the

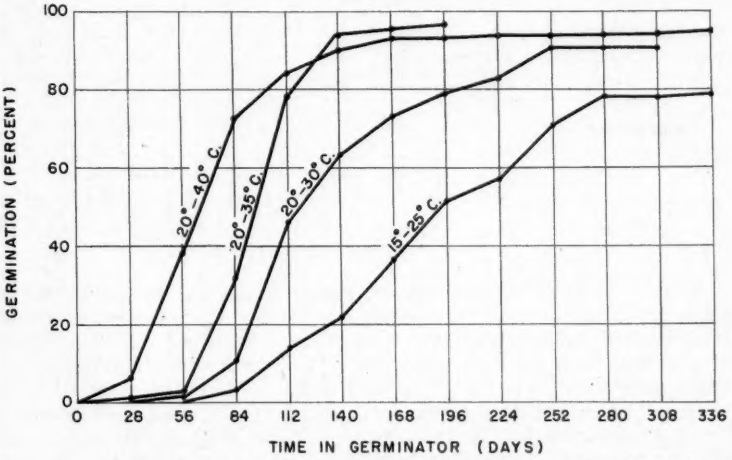

FIGURE 1.—Mean germination of four samples of freshly harvested seed of *Digitaria ischaemum* at four temperature alternations.

remaining seeds continued over a very long period. Even during the period of comparatively rapid germination, the mean rate (1 to 1.5 percent per day) was very slow in comparison with the rate of germination of most crop seeds.

Rapid germination started first at the 20° to 40° C. alternation, about 4 weeks later at 20° to 35°, and after another 3 or 4 weeks at 20° to 30°. When well started, germination was for a time at about the same rate for these three conditions. Although germination started much later at the 20° to 35° than at the 20° to 40° alternation, a smaller percentage of seed remained ungerminated at the former temperature alternation when the rate of germination slowed down in the later part of the test. At the conclusion of most of the individual tests at 20° to 40° a few apparently sound seeds remained, and this in general was not true at 20° to 35°. At 20° to 30° the rate of germination fell markedly after about 50 percent of the seeds had germinated. The difference in germination among samples was greater at 20° to 30° than at 20° to 40° or at 20° to 35°.

TABLE 1.—*Summary of course of germination of 4 samples of Digitaria ischaemum at 4 temperature alternations when tested immediately after harvest*

Temperature alternation (°C.)	Sample No.	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days—											
				28	56	84	112	140	168	196	224	252	280	308	336
			No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
20 to 40	761848	1936	200	0	54	87	93	94	95	95	....	....	....	....	....
	766396	1937	200	11	36	76	89	93	94	95	96	....	....	....	....
	766401	1937	200	15	41	66	79	87	94	95	....	....	....	....	....
	769356	1938	400	0	21	64	74	87	88	89	90	91	91	92	93
Total or mean			1,000	6	38	73	84	90	93	93	94	94	94	94	95
20 to 35	761848	1936	400	0	0	30	78	93	94	95	....	....	....	....	....
	766396	1937	200	2	5	35	87	95	96	97	....	....	....	....	....
	766401	1937	200	1	5	28	73	96	98	99	....	....	....	....	....
	769356	1938	400	0	1	38	74	92	97	97	....	....	....	....	....
Total or mean			1,200	1	3	32	78	94	96	97	....	....	....	....	....
20 to 30	761848	1936	200	0	0	6	30	47	55	59	60	87	88	89	....
	766396	1937	200	0	3	20	51	59	73	79	84	....	....	....	....
	766401	1937	200	1	2	13	51	72	77	89	89	95	....	....	....
	769356	1938	400	0	0	6	54	73	86	90	98	....	....	....	....
Total or mean			1,000	0	1	11	46	63	73	79	83	91	91	91	....
15 to 25	761848	1936	200	0	0	1	8	12	30	47	48	91	91	92	....
	766396	1937	200	0	0	2	8	13	34	40	46	50	64	....	....
	766401	1937	200	0	0	1	7	17	35	63	73	80	93	....	....
	769356	1938	400	0	0	7	33	45	51	58	63	63	64	64	65
Total or mean			1,000	0	0	3	14	22	37	52	57	71	78	78	79

At the 15° to 25° C. alternation, germination was not only slow to start but it progressed at a much slower rate than under the other temperature conditions and the final germination was less. The means of the four samples indicate a fairly uniform rate of germination, approximately 2.75 percent per week from the twelfth to the fortieth week. The total germination obtained at the 15° to 25° alternation was very different for the four samples. This may in part have been due to undetected differences in germination conditions in the different years. On the other hand, samples 766396 and 766401 were in the same chambers at the same time and yet germinated very differently at this temperature alternation, although these same samples behaved essentially alike at 20° to 40° and at 20° to 35°.

STORED SEED

Germination tests started September 23, 1935 (day of seed collection), November 22, 1935, January 13, 1936, and September 30, 1936, on sample 758522, gave results (fig. 2) for the temperature alternation 20° to 35° C. that indicated a progressively earlier start of germination for the successively later tests. When the seed was a year old, germination was completed in 7 days, whereas with freshly harvested seed germination extended over a period of 224 days.

With seed collected in 1936, tests in September and again in December indicated a similar change in the response of the seed. In 1938 it was planned to follow closely the changing germination response of the seed when stored dry in the laboratory. Tests were started (sample 769356) the day the seed was collected, September 8, and at weekly

intervals thereafter at 20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°. By October it was realized that the interval between tests was too short to indicate definite change, but the supply of seed was so nearly exhausted that the series could not be extended. The remaining seed was tested on February 2, 1939. The results of these tests (table 2) suggest that at 20° to 30° and at 20° to 35° the seed germinated better when tested the day it was collected than when tested 1 and 2 weeks later; however, the differences are not large enough or consistent enough to be more than suggestive. With this possible exception the results from September 8 through October 6 for 15° to 25° and 20° to 30°, and through October 13 for 20° to 35° and 20° to 40°, are fairly uniform and show at least no marked change in the response of the

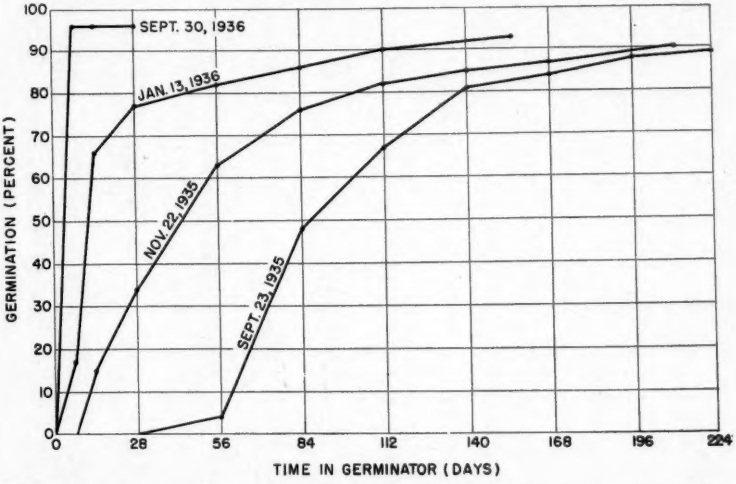

FIGURE 2.—Progress of germination of seed of *Digitaria ischaemum* at 20° to 35° C. alternation when tested at time of harvesting and at different dates thereafter. Sample 758522, collected September 23, 1935.

samples over this period. In February 1939 germination was greater in 14 days than in 112 days for the earlier tests and there was very little difference in the course of germination at the three temperature alternations 20° to 40°, 20° to 35°, or 20° to 30°.

With seed 1 to 3 years old (sample 758522) germination was essentially the same at the four temperature alternations except for slightly slower germination at 15° to 25° C. With seed 1 year old germination at 20° to 40°, 20° to 35°, and 20° to 30° was approximately 95 percent in 7 days and was completed (96 to 99 percent) in 14 days. At 15° to 25° germination was approximately 95 percent in 14 days and was completed in 14 to 21 days. Plumules were noticeably shorter in 5 days at 20° to 40° than at 20° to 35°. There was no evidence of further change in response of the seed after 1 year.

TABLE 2.—*Summary of germination at different temperature alternations of seed of Digitaria ischaemum when tested at successive periods after harvest*

[Sample 769356, collected Sept. 8, 1938. 400 seeds used for each test]

Temperature (° C.)	Period of germination count	Germination [1] in tests started—							
		Sept. 8, 1938	Sept. 15, 1938	Sept. 22, 1938	Sept. 29, 1938	Oct. 6, 1938	Oct. 13, 1938	Mean	Feb. 2, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 40	14	0	0	0	0	1	0	0	77
	28	0	1	0	2	2	1	1	84
	56	21	13	17	22	25	14	19	87
	84	64	58	48	48	55	56	55	87
	112	74	74	68	63	74	76	72	89
	140	87	89	87	80	90	93	88	90
	168	88	93	91	88	91	93	91	96
	196	89	94	94	93	93	95	93	[1] 97 (3)
	224	90	97	95	93	93	95	94	.....
	252	91	97	95	93	93	95	94	.....
	280	91	97	95	94	93	95	94	.....
	308	92	97	95	94	94 (2)	96	95	.....
	336	93 (2)	97 (1)	97	95 (1)	.....	.....	95	.....
20 to 35	14	0	0	0	0	0	1	0	73
	28	0	0	0	0	0	1	0	83
	56	1	0	0	0	1	2	1	86
	84	38	29	22	27	31	41	31	88
	112	74	69	61	67	66	77	69	89
	140	92	90	84	91	91	92	90	89
	168	97	97	93	93	95	97	95	92
	196	97	.....	95	98	98	98	97	94 (3)
20 to 30	14	0	0	0	0	0	.....	0	63
	28	0	0	0	0	0	.....	0	81
	56	0	0	0	0	0	.....	0	84
	84	6	1	1	4	8	.....	4	87
	112	54	38	36	45	45	.....	44	89
	140	73	54	54	62	64	.....	61	92
	168	86	63	63	69	68	.....	70	93
	196	90	67	72	77	77	.....	77	93 (5)
	224	98	77	81	93	91	.....	88	.....
	252	.....	84	84	94	92	.....	91	.....
	280	.....	84	85	94	92	.....	91	.....
	308	.....	84	85	94	93 (5)	.....	91	.....
	336	.....	84 (13)	85 (13)	94 (4)	.....	.....	91	.....
15 to 25	28	0	0	0	0	0	.....	0	.....
	56	0	0	1	2	3	.....	1	.....
	84	7	6	11	10	16	.....	10	.....
	112	33	34	36	37	28	.....	34	.....
	140	45	46	45	51	43	.....	46	.....
	168	51	53	51	56	48	.....	52	.....
	196	58	56	54	60	53	.....	56	.....
	224	63	62	59	66	55	.....	61	.....
	252	63	69	65	69	60	.....	65	.....
	280	64	71	66	70	60	.....	66	.....
	308	64	71	66	73	62	.....	67	.....
	336	65	71	67	73	.....	.....	68	.....

[1] Figures in parentheses after final percentages show approximate percentage of apparently sound seed remaining when test was discontinued.

PRECHILLED SEED

In studying the effect of prechilling, the seeds were placed on moistened paper toweling in Petri dishes, and these were held in a cold chamber at approximately 3° C. (2° to 5°) for various periods before they were removed to the usual germination chambers. The germination count was reckoned from the time the seed was moistened and placed at the low temperature. No germination occurred while the seeds were at the low temperature.

A few representative tests are presented from among the many carried out. The course of germination of freshly harvested seed at 20° to 35° C. after prechilling is shown in figure 3 for two samples collected in different years.

The freshly harvested seed of the two samples behaved very much alike. Prechilling for 2 weeks caused a small proportion of the seeds to germinate more quickly, but after these had germinated the further progress of germination of the prechilled and nonchilled tests was similar, and there was a definite suggestion of a lower final germination of the seed that had been prechilled. Treatment of the freshly harvested seed at a low temperature for 4 weeks caused a much larger proportion of the seeds to germinate rather promptly after they were subjected to a 20° to 35° C. alternation of temperature, but even after this period of treatment a certain proportion of the

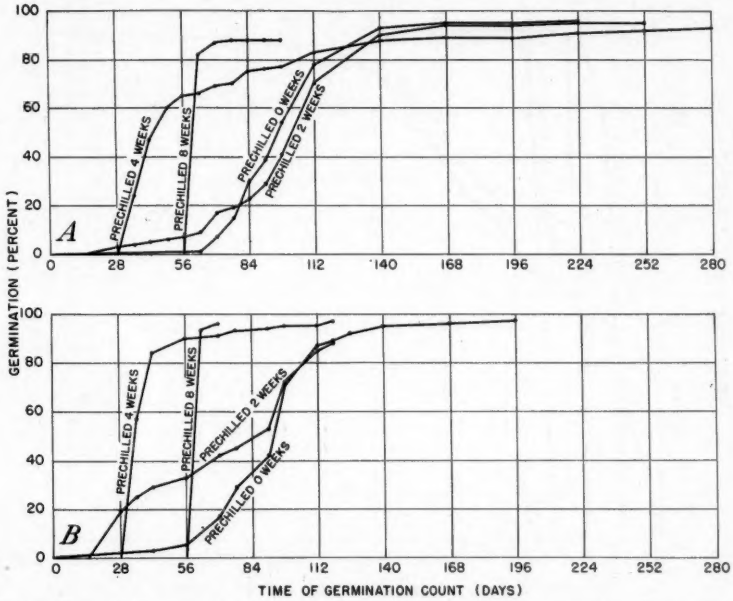

FIGURE 3.—Progress of germination of freshly harvested seed of *Digitaria ischaemum* at 20° to 35° C. after prechilling at 3° for 0, 2, 4, and 8 weeks. *A*, Sample 761848, collected September 28, 1936; tests started September 30, 1936. *B*, Sample 766396, collected September 28, 1937; tests started September 29, 1937.

seeds was at least as slow to germinate as the untreated seeds. After prechilling for 8 weeks, over 90 percent of the seeds germinated within a week after removal to the higher temperature condition, and germination of the viable seeds was completed much earlier than that of the untreated seed.

When the behavior of fresh seed is compared with that of seed from the same sample after approximately 2 months' dry storage (figs. 3, *A;* 4), it is noted that the older seed after 2 weeks' prechilling germinated almost as promptly as the fresh seed after 8 weeks' treatment, although, even with this older seed, a longer treatment (4 weeks) at a low temperature brought about prompt germination of a slightly greater proportion of the seeds.

The seed collected September 8, 1938 (sample 769356), showed, as previously noted, no definitely significant difference in behavior when tested without prechilling at weekly intervals from September 8 to October 13. However the results of tests made at these same intervals with seed that had been prechilled for 2 weeks after dry storage for 0 to 5 weeks (table 3) showed a progressively greater germination at the earlier germination counts as the seed became older. The germination in 28 days of seed that had been stored dry for 4 weeks was 44 percentage units higher than that of the seed tested after 3 weeks in dry storage. Seed that had been stored 5

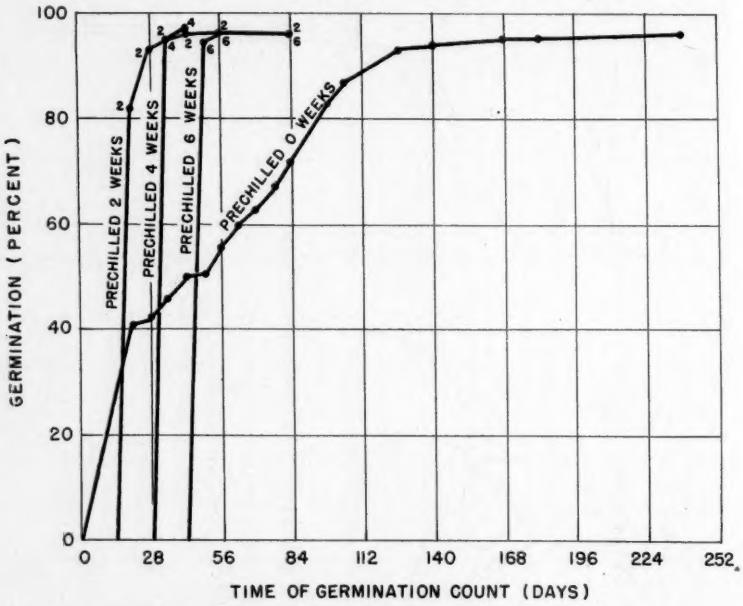

FIGURE 4.—Progress of germination of older seed of *Digitaria ischaemum* at 20° to 35° C. after prechilling at 3° for 0, 2, 4, and 6 weeks. Sample 761848, collected September 28, 1936; tests started December 9, 1936.

weeks and then prechilled for 2 weeks germinated almost as promptly as seed that had been stored dry for 21 weeks but not prechilled. These differences had largely disappeared by the one hundred and fortieth day of germination count.

The results stated above on the effect of prechilling have been based on subsequent germination at 20° to 35° C. A comparison of the progress of germination at four temperature alternations after prechilling the seed 2, 4, and 8 weeks (fig. 5) shows that the response of prechilled seed to temperature of germination was similar to that shown by untreated freshly harvested seed. However, the differences in germination response at the four alternations were less with the longer periods of prechilling.

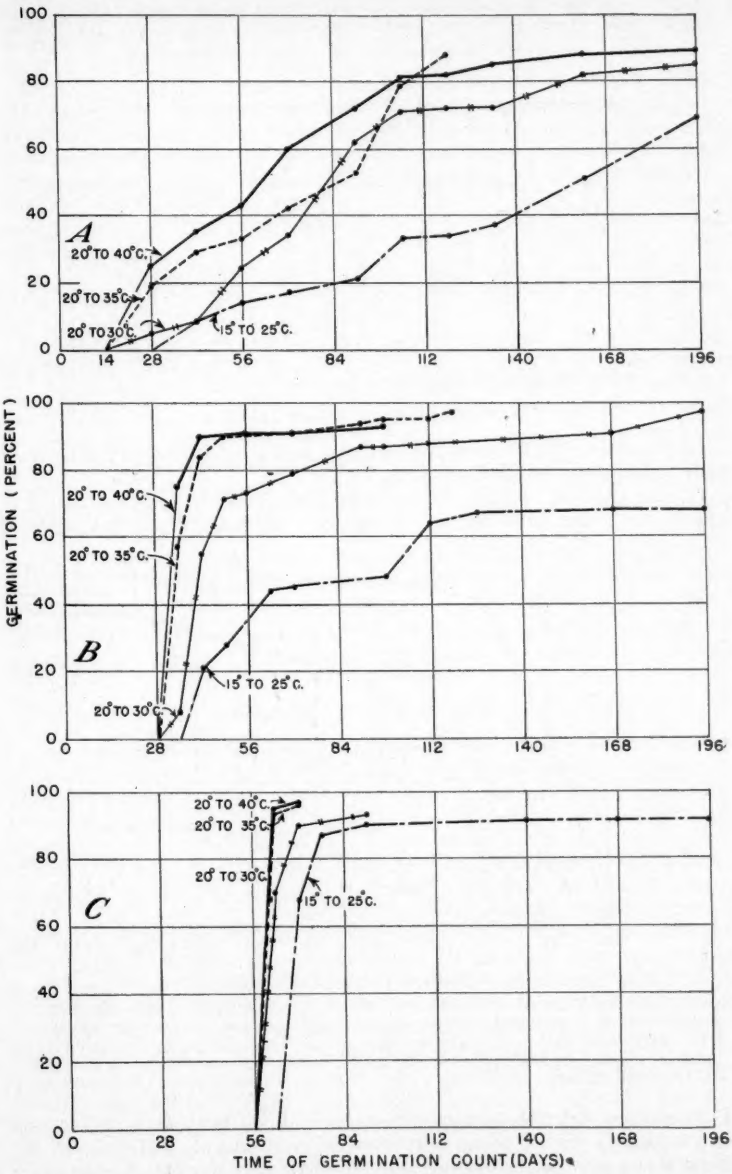

FIGURE 5.—Progress of germination of seed of *Digitaria ischaemum* at four temperature alternations after prechilling at 3° C. for (*A*) 2 weeks, (*B*) 4 weeks, and (*C*) 8 weeks. Sample 766396, collected September 28, 1937; tests started September 29, 1937.

TABLE 3.—*Effect of prechilling at 3° C. for 2 weeks on the subsequent germination at 20° to 35° of seed of Digitaria ischaemum stored dry for 0, 1, 2, 3, 4, or 5 weeks*

[Sample 769356, collected Sept. 8, 1938. 400 seeds used for each test]

Period of germination count (days)	Germination after prechilling for 2 weeks at 3° C. after dry storage for—						Germination without prechilling after dry storage for—	
	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks	0–5 weeks [1]	21 weeks
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
28	8	8	17	31	75	76	0	83
56	16	13	27	51	80	80	1	86
84	36	29	52	68	81	81	31	88
112	64	66	67	75	82	83	69	89
140	85	89	83	93	88	91	90	89
168	93	95	95	96	94	97	95	92
196	95	98	98	99	99	97	97	94

[1] Means of the 6 tests started at weekly intervals from Sept. 8 to Oct. 13, inclusive.

MISCELLANEOUS OBSERVATIONS

In the course of the above-described work other observations were made that seem worth recording.

In the general routine of testing, additional tests were made in which the paper toweling was moistened with a 0.2-percent solution

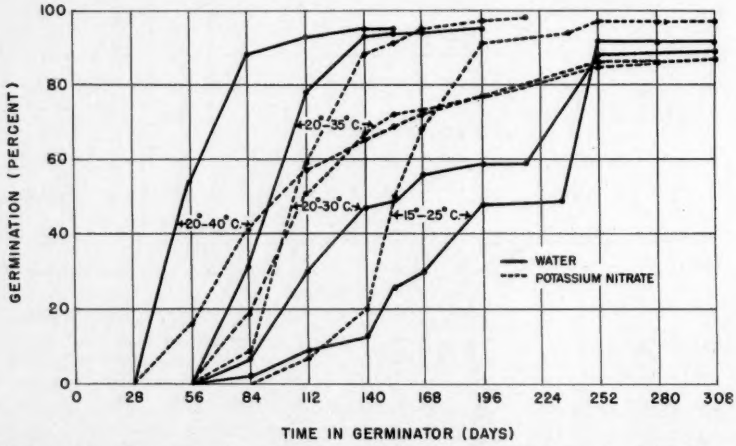

FIGURE 6.—Progress of germination of freshly harvested seed of *Digitaria ischaemum* at four temperature alternations, on paper toweling moistened with water and with a 0.2-percent solution of potassium nitrate at each alternation. Means of 200 seeds for each test, except 400 seeds used at 20° to 35° C. Sample 761848.

of potassium nitrate instead of water. The most striking results were obtained with freshly harvested seed of sample 761848 (fig. 6). There was a distinct hastening of germination by the use of potassium nitrate at the 15° to 25° C. and 20° to 30° temperature alternations, and an equally strong retardation at the 20° to 35° and 20° to 40° alternations.

Difference in germination between seeds treated with water and

seeds treated with potassium nitrate was not apparent at some of the temperature alternations with some samples, but whenever a difference occurred it was in the direction indicated in figure 6.

A detailed study of the effect of light on germination was not made. Most of the tests received some light from electric lamps used to heat the germination chambers and also from brief exposure during transfer from one temperature to another and during the counting of the germinated seedlings. Some of the series received definite exposure to diffuse daylight during a portion of each day. In a few instances there were parallel tests in which direct light exposure was compared with total light exclusion by enclosing the tests in a tin box. The results were somewhat erratic and inconclusive, and no definite or striking effect of light on the germination of seed of *Digitaria ischaemum* was observed.

It is usually assumed that retarded germination such as reported here for *Digitaria* is caused by restrictions of the seed or fruit coats; therefore, some tests were conducted to determine the effect of mechanical scarification and treatment with concentrated sulfuric acid. Rubbing the fruits with emery paper caused a slight hastening of germination in the first few weeks, but about two-thirds of the seeds were badly injured by the treatment. After treatments for ½ minute and for 1 minute with concentrated sulfuric acid, approximately 60 percent of the seed germinated in 28 days, at which time germination had hardly started in the control. After 19 weeks in the germinator there was very little difference between the germination of the treated and the untreated seeds. Treatment for 2 minutes caused marked injury.

EFFECT OF SEVERAL TEMPERATURE ALTERNATIONS ON GERMINATION OF SEED OF DIGITARIA SANGUINALIS

Freshly Harvested Seed

A summary of the germination of several samples of seed of *Digitaria sanguinalis* collected in different years and tested within a few days after harvest at several temperature alternations is given in table 4. Germination of the several samples at any one of the temperature alternations was very variable. This variation is evident in figure 7, which shows the progress of germination at four temperature alternations of two samples collected the same year and tested at the same time. (See also fig. 11.) In spite of this variation among samples, the relative response of each of the samples to the different temperature conditions was much the same (table 4). The progress of germination at different temperature alternations, based on the means of all available information for each alternation, is shown in figure 8.

In general, germination was most rapid and reached the highest final value at 20° to 30° C. with light. Germination at 20° to 35° progressed at a somewhat slower rate, and final values were slightly lower. At 20° to 40° the start of germination was delayed and its progress was at a definitely slower rate than at the two conditions mentioned above, and for most of the samples germination of the viable seed was not completed in the time the tests were conducted. Germination of three samples tested at 15° to 25° was extremely slow. The behavior of the freshly harvested seed at the other alternations used will be discussed later, in connection with the effect of exposure to light (p. 83).

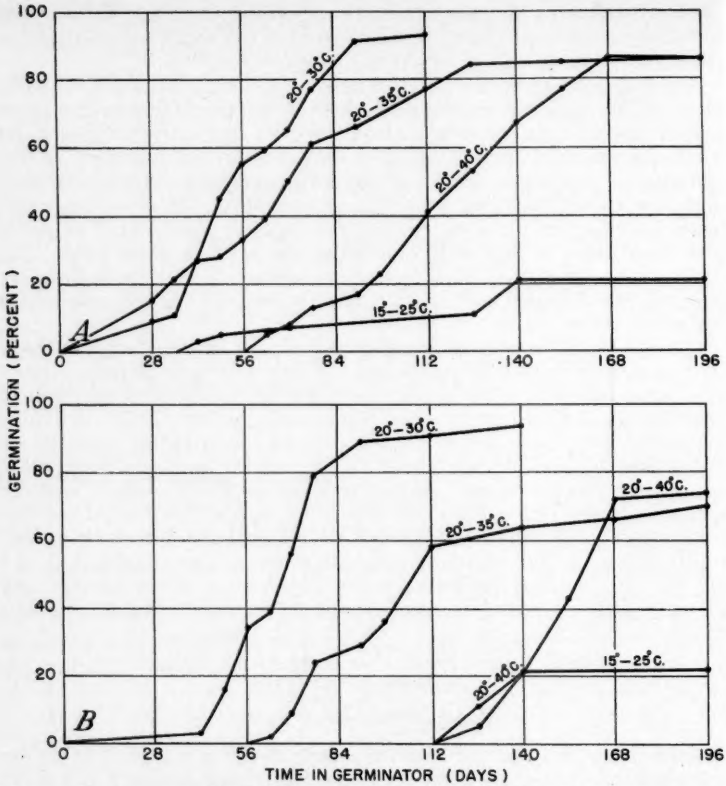

FIGURE 7.—Progress of germination of freshly harvested seed of *Digitaria sanguinalis* at four temperature alternations, showing variable response of (*A*) sample 766403 and (*B*) sample 766404. Seed collected September 29, 1937; germination started October 2, 1937.

TABLE 4.—*Course of germination of various samples of seed of Digitaria sanguinalis at different temperature alternations when tested immediately after harvest*

Temperature alternation (°C) and light condition.	Sample No.	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days											
				28	56	84	112	140	168	196	224	252	280	308	336
			Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 40	761849	1936	200	0	16	43	60	80	83	83	85	86	86	92	----
	766403	1937	100	0	3	15	41	67	86	86	86	----	----	----	----
	766404	1937	200	0	0	0	2	21	72	74	75	----	----	----	----
	769357	1938	400	0	2	11	33	54	61	65	73	74	74	74	75
Total or mean	----	----	900	0	5	17	34	55	75	77	80	80	80	82	----
20 to 35	758523	1935	200	0	33	62	87	96	97	----	----	----	----	----	----
	761849	1936	400	0	29	72	84	89	91	91	91	94	----	----	----
	766403	1937	100	15	33	63	77	84	85	86	86	----	----	----	----
	766404	1937	200	0	0	26	58	64	66	70	70	----	----	----	----
	769357	1938	400	0	14	55	76	84	86	86	88	91	91	93	93
Total or mean	----	----	1,300	3	22	56	76	83	85	86	87	88	88	88	----

TABLE 4.—*Course of germination of various samples of seed of Digitaria sanguinalis at different temperature alternations when tested immediately after harvest*—Cont.

Temperature alternation (°C) and light condition.	Sample No.	Year of harvest	Seeds tested	Cumulative percentage germination after indicated number of days											
				28	56	84	112	140	168	196	224	252	280	308	336
			Number	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
20 to 30, with light	758523	1935	200	1	26	89	92	92							
	761849	1936	200	0	37	84	92	92	92	94	95	99			
	766403	1937	100	9	55	83	93								
	766404	1937	200	1	34	84	91	94							
	769357	1938	400	0	7	85	86	89	90	90	91	94	95	97	97
Total or mean			1,100	2	32	85	91	92	92	93	93	95			
20 to 30, light excluded	761849	1936	200	0	3	4	17	21	25	26	28	45	51		
Room to 35	758523	1935	200	0	35	81	91	94							
	761849	1936	200	2	65	87	90	92	96						
Total or mean			400	1	50	84	90	93	95						
15 to 25	761849	1936	200	0	0	0	0	0	1	2	2	20	22	23	
	766403	1937	100	0	5	7	7	21	21	21					
	766404	1937	200	0	0	0	3	21	21	22	22				
Total or mean			500	0	2	2	3	14	14	15	15	21			

STORED SEED

The course of germination at 20° to 30° C. with light in successive tests of sample 758523, collected September 23, 1935, and tested 1 day, 2 months, 3½ months, and 1 year after harvest, is shown in figure 9. In 1938 sample 769357 was placed to germinate on the day it was harvested and after 8 intervals of approximately 1 month each. A summary of the complete results is presented in table 5, and the course of germination for some of the tests at 20° to 30° C. with light is shown

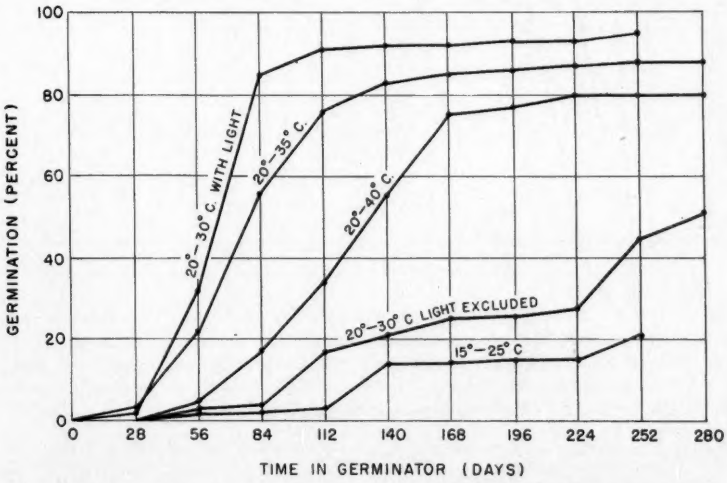

FIGURE 8.—Mean germination of fresh seed of *Digitaria sanguinalis* for all years, showing the progress of germination at the various temperature alternations.

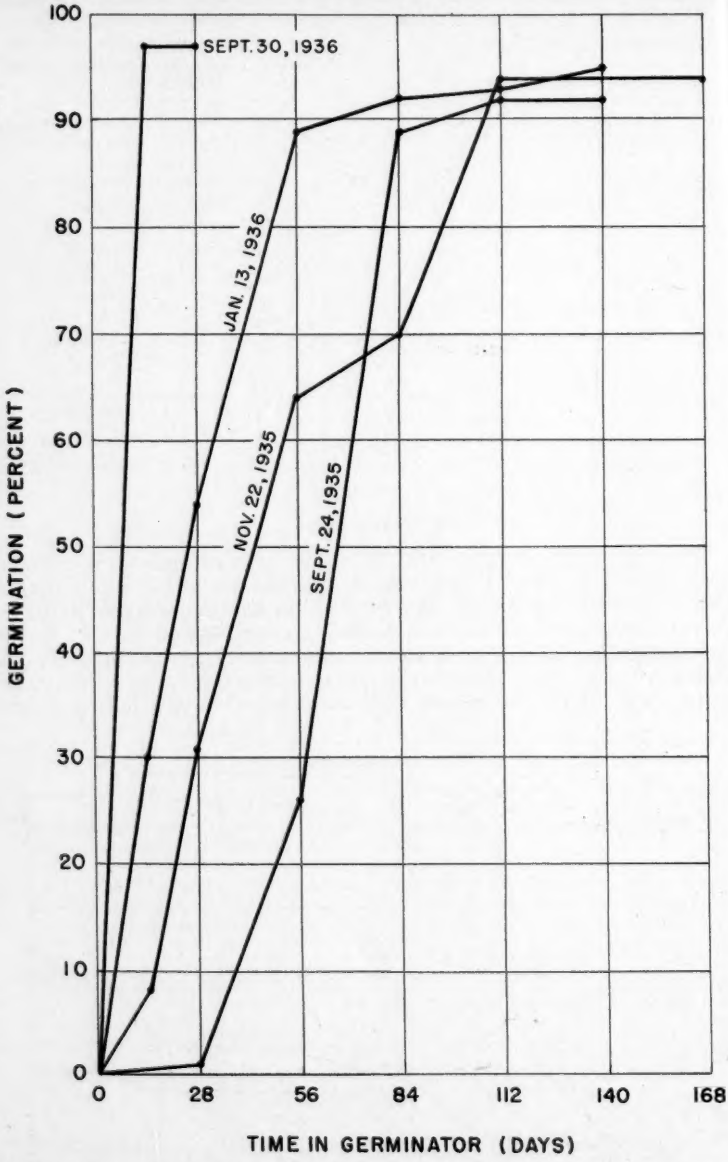

FIGURE 9.—Progress of germination of seed of *Digitaria sanguinalis* at 20° to 30° C. alternation with light, at different dates after harvesting. Sample 758523, collected September 23, 1935.

TABLE 5.—*Summary of germination at different temperatures of seed of Digitaria sanguinalis when tested at successive periods after harvest*

[Sample 769357, collected Sept. 8, 1938. 400 seeds used for each test]

Temperature alternation (°C.) and light condition	Period of germination count	Germination [1] in tests started—								
		Sept. 8, 1938	Oct. 6, 1938	Nov. 3, 1938	Dec. 1, 1938	Jan. 5, 1939	Feb. 2, 1939	Mar. 2, 1939	Mar. 31, 1939	Apr. 28, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 40	14	0	0	0	5	30	42	40	72	70
	28	0	1	2	9	35	46	43	74	71
	56	2	3	4	10	39	50	44	76	74
	84	11	8	6	14	41	52	45	77	76
	112	33	39	28	35	45	54	51	78	[2] 77
	140	54	64	59	53	49	63	61	81(19)	
	168	61	76	72	58	58	75	68(30)		
	196	65	80	75	60	68	77(21)			
	224	73	80	75	65	70				
	252	74	80	77	66					
	280	74	81	78						
	308	74	81							
	336	75								
20 to 35	14	0	0	2	27	36	64	78	86	90
	28	0	0	14	36	54	73	84	91	92
	56	14	35	39	47	63	79	85	93	94
	84	55	57	54	60	68	80	87	93	94
	112	76	78	75	71	73	83	88	93	94(5)
	140	84	86	80	78	78	84	90	93(6)	
	168	86	87	84	81	80	88	91(8)		
	196	86	90	86	81	86	88(10)			
	224	88	92	86	83	87(9)				
	252	91	92	89	85(12)					
	280	91	92	90(8)						
	308	93	93(5)							
	336	93(1)								
20 to 30 without definite light exposure	14	0	0	1	16	29	58	55	62	83
	28	0	4	31	45	52	74	75	81	87
	56	21	38	63	62	64	84	80	84	90
	84	54	58	62	68	65	87	87	85	91
	112	73	73	75	70	66	89	87	86	91(9)
	140	77	66	75	71	72	90	88	88(12)	
	168	79	66	76	74	75	91	88(10)		
	196	79	67	79	77	77	91(8)			
	224	79	71	80	81	78(20)				
	252	85	73	84	85(13)					
	280	87	81	85(14)						
	308	91	81(14)							
	336	92(4)								
20 to 30 with light	14	0	0	0	6	29	33	48	71	93
	28	0	2	16	38	54	74	78	90	96
	56	7	65	75	67	82	87	90	95	97
	84	85	72	88	74	86	93	95	96	98
	112	86	84	89	83	90	95	95	97	99(0)
	140	89	85	91	84	93	95	96	97(1)	
	168	90	85	91	90	93	95	97(1)		
	196	90	86	93	91	93	97(1)			
	224	91	93	95	93	97(1)				
	252	94	95	97	95(3)					
	280	95	96	98						
	308	97	97(2)							
	336	97(2)								

[1] Figures in parentheses after final percentage show percentage of apparently sound seed remaining when test was discontinued.

[2] Many apparently sound seed remaining when test was discontinued.

graphically in figure 10. With both of these samples the germination in 28 days was progressively greater as the seed aged, but the germination finally obtained was about the same, whatever the age of the seed. Seed of sample 769357 (table 5) placed to germinate on April 28, about 6¾ months after collection, showed about the same germination in 28 days as the freshly harvested seed in 252 days. The change in rate of germination is also shown in the part of figure 14 that presents tests without prechilling. The relation of time since harvest to germination is brought out more clearly by a retabulation of the germination results at 20° to 30° C. with light on the basis of the actual

date of the germination count (table 6). Germination on May 25 was essentially the same whether the seeds had been moist in the germinator all the time since the seed was harvested, or had been

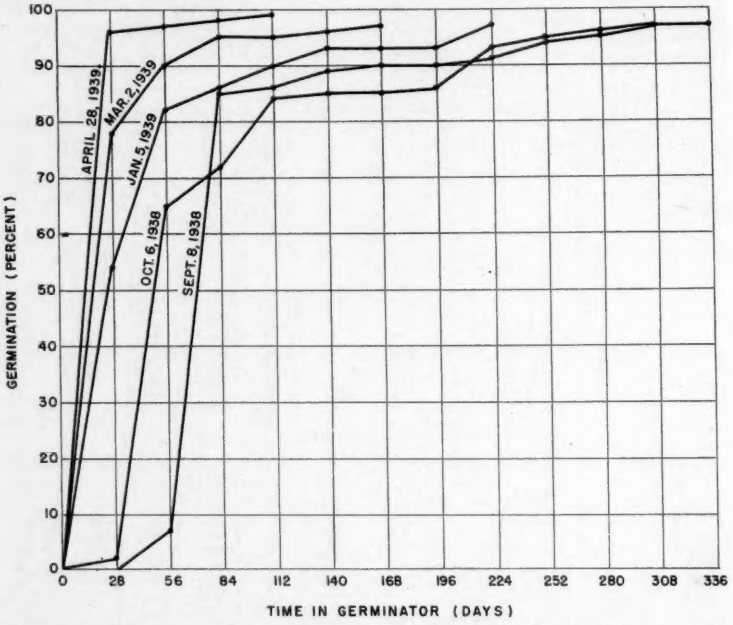

FIGURE 10.—Progress of germination of seed of *Digitaria sanguinalis* at 20° to 30° C. alternation with light, tested at successive dates after harvesting. Sample 769357, collected September 8, 1938.

stored dry for different portions of the time. Also, germination was practically completed (95 to 99 percent) by August 17 irrespective of the date that germination started.

TABLE 6.—*Germination of seed of Digitaria sanguinalis when tested at successive periods after harvest, at 20° to 30° C. alternation with light, based on the actual date of germination count*

[Sample 769357, collected Sept. 8, 1938]

Date of germination count	Germination in tests started—								
	Sept. 8	Oct. 6	Nov. 3	Dec. 1	Jan. 5	Feb. 2	Mar. 2	Mar. 31	Apr. 28
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
May 25	95	94	94	90	93	95	95	95	96
June 23	96	96	97	92	93	95	95	96	97
July 20	97	97	97	94	93	95	96	97	98
Aug. 17	98	97	98	95	97	97	97	97	99

After approximately 8 months' storage, the differences in rate of germination at the different temperature alternations (fig. 11) had largely disappeared. A great part of the total germination at each

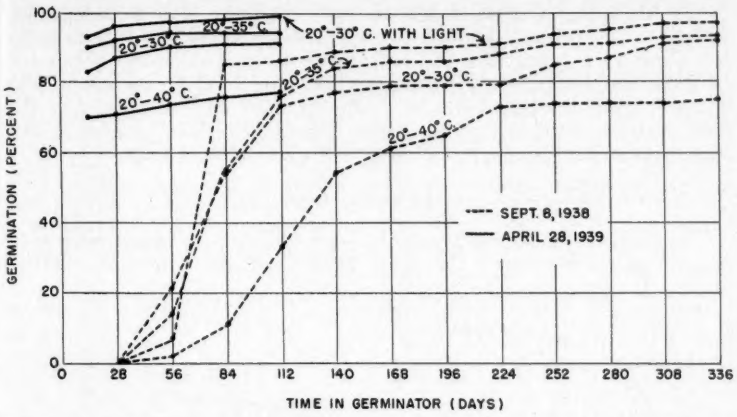

FIGURE 11.—Progress of germination of seed of *Digitaria sanguinalis* at different temperature alternations, tests at harvesting and after storage for 8 months. Sample 769357, collected September 8, 1938.

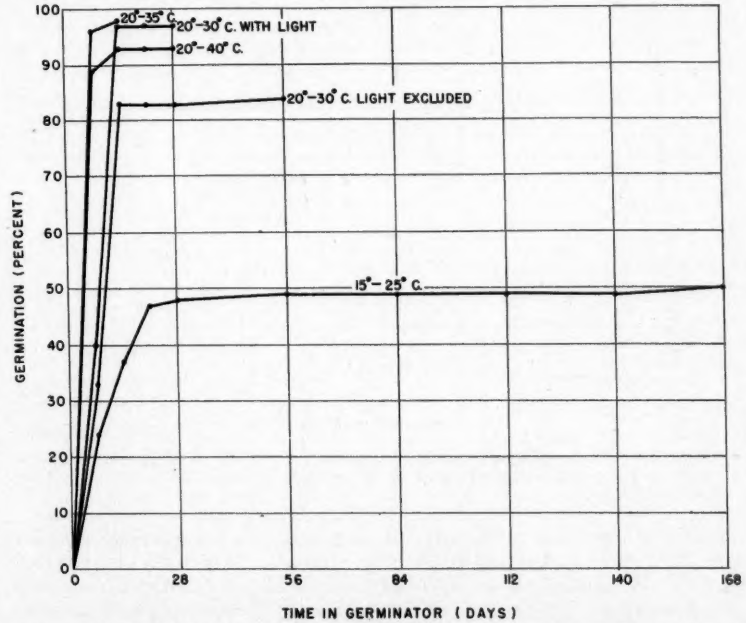

FIGURE 12.—Progress of germination of seed of *Digitaria sanguinalis* at different temperature alternations after storage for 1 year. Sample 758523.

alternation was in the first 14 days. However, the total germination at 20° to 40° C. was distinctly lower than at the other alternations. Germination of seed after 1 year of storage (sample 758523, fig. 12) was more than 90 percent and was practically completed in 14 days

at 20° to 40° as well as at 20° to 30° with light, and at 20° to 35°. At 15° to 25° (sample 758523, fig. 13), the 1-year-old seed germinated less than 50 percent and the 2-year-old seed less than 80 percent in 56 days. It was not until the seed had been held in dry storage for 3 years that germination was fairly prompt and complete at this temperature, although at the other germination temperatures used there was no further change in response after the first year.

Prechilled Seed

The results of germination of the seed of *Digitaria sanguinalis* after prechilling were erratic. Sample 758523, collected in 1935, showed

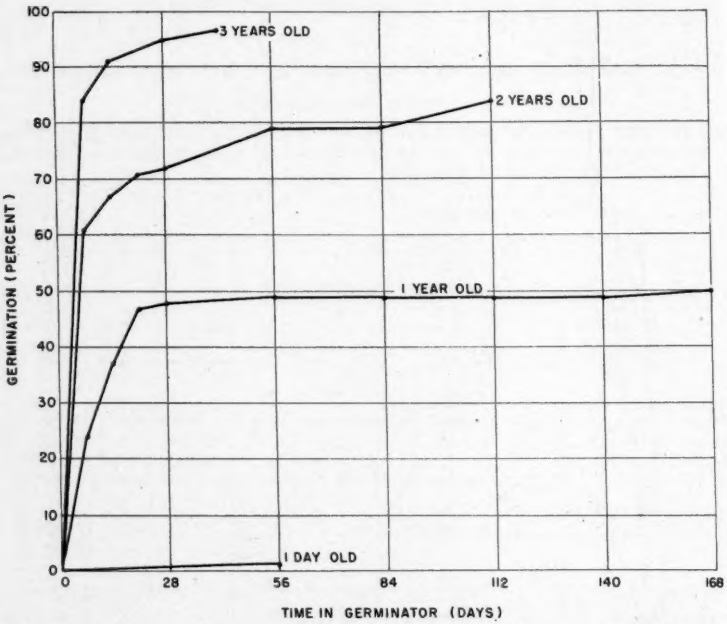

Figure 13.—Change of germination response at 15° to 25° C. of seed of *Digitaria sanguinalis* stored over a period of 3 years with tests at yearly intervals. Sample 758523.

essentially the same percentage of germination after prechilling for 1 and for 2 weeks as without such treatment. When the seed was 2 months old, prechilling for 2 weeks brought about a distinct hastening of germination, but treatment for 4, 8, and 12 weeks caused distinct injury, resulting in progressively lower percentages of final germination as the period of chilling was lengthened.

The results of prechilling on sample 761849, collected in 1936, are presented in table 7. Prechilling the freshly harvested seed for 2 and for 4 weeks caused a small but definite increase in early germination, but there was a distinct tendency for the later germination to fall below that of the control. The older seed was definitely stimulated

by prechilling for 2 and 4 weeks. In both instances there was definite injury from treatments longer than 4 weeks.

TABLE 7.—*Effect of prechilling at 3° C. on germination at 20° to 35° alternation of seed of Digitaria sanguinalis when tested immediately after harvest and about 2 months later*

[Sample 761849, collected Sept. 28, 1936. 200 seeds used in each test]

Period of germination count (days)	Germination after prechilling for indicated number of weeks in tests started Sept. 30, 1936				Germination after prechilling for indicated number of weeks in tests started Dec. 9, 1936			
	0	2	4	8	0	2	4	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
28	0	1	----	----	78	89	----	----
56	29	47	70	----	80	92	92	84
84	72	75	79	69	83	93	92	85
112	84	79	81	70	86	93	92	----
140	89	84	84	71	86	93	93	----
168	91	85	85	71	86	----	94	----
196	91	85	85	71	89	----	94	----
224	91	85	85	72	91	----	94	----
252	94	86	86	----	----	----	----	----
280	----	88	87	----	----	----	----	----
308	----	90	89	----	----	----	----	----

The results of prechilling seed collected in 1938 (sample 769357) at monthly intervals after harvest are given in table 8. The relative increase in the early germination caused by prechilling for 4 weeks is shown in figure 14, based on germination in 56 days (tables 5 and 8).

TABLE 8.—*Summary of germination of seed of Digitaria sanguinalis, after prechilling at 3° for 4 weeks, when tested at successive periods after harvest*

[Sample 769357, collected Sept. 8, 1938. 400 seeds used in each test]

Temperature alternation (° C.)	Time of germination counts	Germination [1] in tests started—								
		Sept. 8, 1938	Oct. 6, 1938	Nov. 3, 1938	Dec. 1, 1938	Jan. 5, 1939	Feb. 2, 1939	Mar. 2, 1939	Mar. 31, 1939	Apr. 28, 1939
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20 to 30	35	3	11	42	56	72	68	72	77	73
	42	18	56	78	82	90	85	87	93	92
	56	52	83	86	89	92	91	93	95	94
	84	76	85	89	90	92	96	94	96	96
	112	79	85	89	90	92	96	94	96	96(2)
	140	82	85	89	90	92	96	94	96(2)	----
	168	82	85	90	91	92	96	94(4)	----	----
	196	83	89	93	91	92	96(2)	----	----	----
	224	85	94	94	92	92(5)	----	----	----	----
	252	87	95	95	92(8)	----	----	----	----	----
	280	87	96	95(2)	----	----	----	----	----	----
	308	88	96(3)	----	----	----	----	----	----	----
	336	88	----	----	----	----	----	----	----	----
	343	89(1)	----	----	----	----	----	----	----	----
20 to 35	35	9	34	71	69	80	85	87	93	82
	42	33	73	92	95	93	96	95	97	96
	56	62	89	94	97	96	97	98	98	97
	84	79	95	94	98(0)	96	99	98	98(0)	98(0)
	112	82	95	95	----	97	99	98	----	----
	140	85	96	95	----	97	99	98	----	----
	168	86	96	95	----	97	99	98(0)	----	----
	196	87	97	95	----	97	99(0)	----	----	----
	224	89	97	95	----	97(1)	----	----	----	----
	252	90	97(0)	95(0)	----	----	----	----	----	----
	280	90	----	----	----	----	----	----	----	----
	308	90	----	----	----	----	----	----	----	----
	336	90	----	----	----	----	----	----	----	----
	343	90(3)	----	----	----	----	----	----	----	----

[1] Figures in parentheses after final percentage show percentage of apparently sound seed remaining when test was discontinued.

By the time the seed was 8 weeks old germination of the seed prechilled 4 weeks was above 90 percent after 28 additional days at 20° to 35° C., whereas germination of the unchilled seed was about 40 percent. The test, started 7 months after harvest, was the first in which seed without prechilling germinated above 90 percent in 56 days. The results in table 8 show that germination after prechilling was quicker

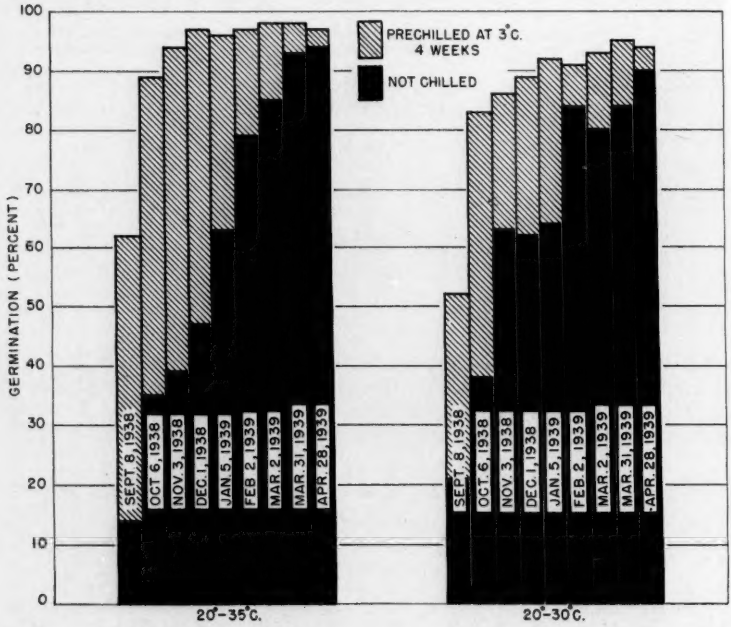

FIGURE 14.—Relative increase in early germination (56 days) of seed of *Digitaria sanguinalis* after prechilling at 3° C. for 4 weeks prior to germinating at two temperature alternations when tested at nine successive approximately monthly intervals. Sample 769357, collected September 8, 1938.

and more complete at 20° to 35° than at 20° to 30°. In both series no definite exposure to light was given, but light was not excluded.

MISCELLANEOUS OBSERVATIONS

In the germination tests of *Digitaria sanguinalis* discussed above, the substratum was moistened with water. In most instances comparable tests were made with the substratum moistened with a 0.2-percent solution of potassium nitrate. The effect of potassium nitrate solution was variable; no definite or consistent effect was observed except a stimulation of mold development even greater than with *D. ischaemum*.

In contrast to the result with *Digitaria ischaemum*, there was a definitely beneficial effect of light on the germination of seed of *D. sanguinalis*. The results in table 4 and figure 8 for freshly harvested seed show that germination of sample 761849 was much faster and better at 20° to 30° C. with light than at the same temperature with

light excluded by enclosing the tests in a tin box. Table 4 also shows faster germination of samples 758523 and 761849 at room temperature to 35° than at 20° to 35°; in this comparison the temperatures for the longer period of the alternation, although similar, are not identical.

The effect of exclusion of light when the seed was tested approximately 3½ months after harvest is shown in figure 15. The benefit of light is marked at 20° to 30° C., but at room temperature to 35° the difference in germination between light and dark is not so great. When this same sample was tested 1 year after harvest (fig. 12) about 80 percent of the seeds germinated equally well in the light or in the dark, but the remainder of the seeds germinated well in the light and very slowly if at all in the dark. The results with sample 769357 tested at intervals after harvest (table 5) show rather consistently an earlier start of germination at 20° to 30° without definite light exposure

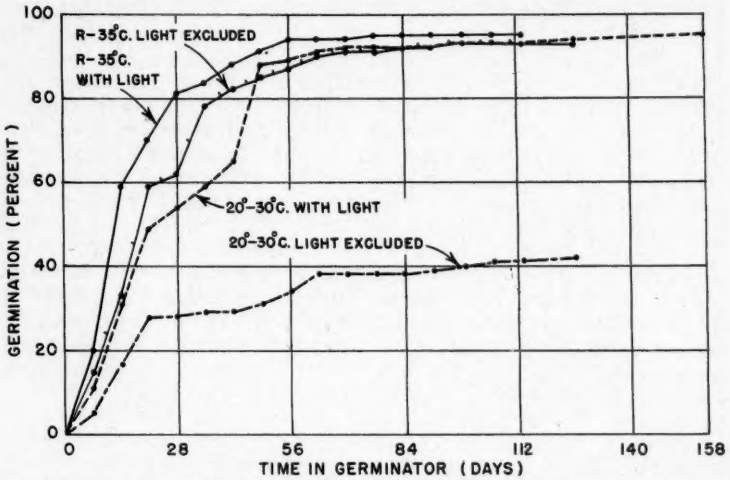

FIGURE 15.—Progress of germination of seed of *Digitaria sanguinalis* at two temperature alternations, with light and with light excluded. Sample 758523, collected September 23, 1935; tests started January 13, 1936.

(except some light from electric lamps used to heat the chambers and some light admitted during transfer of tests and during counting) than at 20° to 30° with light. However, with a definite light exposure, later germination was more rapid and final germination percentages usually were higher. These two series of tests were in entirely different chambers, and differences in humidity and variables other than light may have influenced the results to some extent.

Treatments of seed of one sample with concentrated sulfuric acid for various periods from ½ minute to 3 minutes brought about an earlier start of germination than that which took place without acid treatment.

DISCUSSION

Probably the most striking feature of the germination of these two species of *Digitaria* is the extremely slow but steady germination of the freshly harvested seed under the most favorable conditions. In general

more than 100 days was required for 80 percent of the sample to germinate, and 196 days would seem to be the shortest time in which one could expect completion of germination. In contrast with this extremely slow germination of seed freshly harvested, seed that was 1 year old germinated rather rapidly, germination being completed in 7 to 14 days.

Both species of *Digitaria* germinated very little at the various constant temperatures tried and seemed to require daily temperature alternations. Liebenberg (*11*) in 1884 appears to have been the first to call attention to the value of definite daily alternations of temperature in seed germination. Since then many have shown the need of alternating temperatures for germination of many kinds of seeds.

With freshly harvested seed there was a distinct difference in the response of the two species to the different temperature conditions used. With seed of *Digitaria ischaemum* differences in the length of incubation time before germination started were a striking feature in the response to the temperature alternations 20° to 40° C., 20° to 35°, and 20° to 30°. On the other hand, seed of *D. sanguinalis* did not show much difference in the incubation period, but the rate of germination was markedly affected by germination temperature. The alternation 20° to 40° was distinctly favorable for a large proportion of the seeds of each sample of *D. ischaemum;* but at this alternation seeds of *D. sanguinalis* had a long incubation period, a slow rate of germination, and a low total germination. The alternation 15° to 25° was distinctly unfavorable for both species, but especially so for *D. sanguinalis*. The definitely beneficial effect of light exposure for the germination of *D. sanguinalis* affected the results of the temperature studies with this species. It would appear that the alternations 20° to 35° and 20° to 30° were equally good, but that light was necessary for full germination at either temperature condition. The small amount of light from the lamps used to heat the chambers greatly increased germination over that obtained when light was totally excluded, but it was not sufficient for rapid and complete germination. It is possible that the very low results at 15° to 25° with *D. sanguinalis* were due in part to the small amount of light in the chambers at these lower temperatures.

The beneficial effect of dilute solutions of nitrates usually has been associated with the beneficial effect of light, although many exceptions have been noted in the literature. No explanation is offered for the retarding influence of potassium nitrate on germination of *Digitaria ischaemum* at higher temperatures and the stimulation at lower temperatures, nor for the apparent lack of consistent influence of potassium nitrate on the germination of *D. sanguinalis* although this species is benefited by light exposure.

It has been recognized generally that the seeds of a sample may not respond alike to germination conditions. This fact is illustrated by the present results, especially by the favorable response of a large proportion of the fresh seeds of *Digitaria ischaemum* to the 20° to 40° C. alternation and the decidedly unfavorable response of a small proportion to this temperature condition. This small portion gave a slightly more favorable response to the 20° to 35° alternation. At 20° to 30° the rate of germination began to fall after about one-half of the seeds had germinated. Aside from the long period of incubation before the start of germination, the most striking feature in the progress of

germination of these two species is perhaps the variable proportion of resistant seeds at the different temperature conditions.

Haasis (*6*), Edwards (*5*), and Livingston and Haasis (*12*) have suggested that a given lot of seeds might be separated into physiological classes on the basis of differences in rate of germination. The results presented in this paper indicate that the proportions in the different classes would vary greatly with the temperature conditions of germination.

Both species of *Digitaria* showed a marked change in the progress of germination as the seed aged. This change was evident first as a shortening of the incubation period before germination, and later as a marked increase in the rate of germination. Even at the most favorable temperature condition for each species, a certain proportion of the freshly harvested seed (20 to 40 percent) was very resistant to germination, but as the seed aged this proportion became smaller until at about 8 months after harvest only 5 to 10 percent were resistant. Although seed of *D. sanguinalis* 1 to 3 months old started to germinate much more quickly than fresh seed, there was a suggestion that a small proportion of this slightly older seed was more resistant to germination than the fresh seed, as is shown in figure 10 and table 5.

With many kinds of seeds that germinate readily, the germination response to temperature may be similar to the growth response of the seedling, as would seem to be the case in the results presented by Haasis (*6*) and by Edwards (*5*). On the other hand, many kinds of seeds require special conditions for the initiation of the germination process that may have no close relation to the growth response of the seedling. The difference in the progress of germination at the various temperature conditions of fresh seed of both species of *Digitaria* would indicate that a major effect of germination condition was in controlling preliminary changes in the seed necessary for the initiation of germination. However, the two species responded very differently in this respect to the 20° to 40° C. alternation. As the seed aged it became less sensitive to conditions controlling initiation of germination, so that by the time the seed was a year old germination started promptly under all the temperature conditions used. At this time the small difference in response to temperature observed in the germination of *D. ischaemum* was due to differences in growth rate. The seed of *D. sanguinalis* had mostly afterripened in 1 year when measured by its response to 20° to 40°, 20° to 35°, and 20° to 30° alternations, with light; but at 15° to 25° it was not until the seed was 3 years old that the rate and completeness of germination equaled 1-year-old seed of *D. ischaemum*. A small percentage of the seeds of *D. sanguinalis* required light for germination even after 1 and 2 years' storage.

Prechilling the moist seed at 3° C. brings about a quicker initiation of the germination process and a faster rate of germination. Apparently the preliminary changes that take place slowly in dry stored seed or in seed held under germination conditions are brought about much more quickly in seed held moist at a low temperature. Fresh seed of *Digitaria ischaemum* prechilled for 8 weeks germinated at 20° to 35° almost as promptly as 1-year-old seed without chilling, and the progress of germination of 1-month-old seed after prechilling for 2 weeks was similar to that of 6-month-old seed that had not been prechilled. With fresh seed of *D. ischaemum* a short period of chilling

caused a few seeds to germinate promptly, but the remaining seeds germinated about as slowly as untreated seed and there was some indication that they were slower (fig. 3). As the seed became older or with a longer period of prechilling of fresh seed the proportion of seeds resistant to germination was smaller. Seed stored dry for 1 month and then prechilled for 4 weeks germinated very much like untreated seed that had been stored dry for 6 months longer. The variation in the condition of individual seeds of a sample is indicated by the response to different periods of chilling as well as by the response to different temperature conditions.

The germination results after prechilling were much more definite with seed of *Digitaria ischaemum* than with seed of *D. sanguinalis*. The results with the latter species were affected by a definite tendency for injury to the seeds to occur during long periods of prechilling. It is possible that this injury is related to immaturity of some of the seeds and to the presence of fungi. With the seed of *D. sanguinalis* used and under the conditions of these experiments periods longer than 4 weeks at a low temperature caused injury, and this period of treatment was not long enough to overcome the dormancy of all seeds when tested immediately after harvest.

Davis and Rose (*3*) have shown that prechilling *Crataegus* seed for 2 to 3 months brings about changes in the dormant embryo that initiate normal germination. Toole (*14*) and Whitcomb (*16*) have shown that freshly harvested cereals in which resistance to germination at higher temperatures is caused by the coats will germinate readily at higher temperatures after being prechilled for 5 to 7 days. The response of the seed of *Digitaria* to acid treatment as well as the response to chilling would indicate that the slow germination of fresh seed of these species probably is due both to coat restrictions and to embryo dormancy.

No study of seed structure was made, but Harz (*9*) states that the coat structure of *Digitaria sanguinalis* is similar to that of *Panicum miliaceum*. The latter species is not especially resistant to germination.

In comparing the response of seeds to germination conditions the general progress of germination would seem to be a better guide than the germination attained after any time interval. The optimum condition for germination may, as here, involve many factors, but it would seem to be best expressed as the condition at which germination progresses at the fastest rate and at which germination of the viable seeds is completed in the shortest time.

Although the seeds of these two species of *Digitaria* show some differences in their response to temperature, light, and dilute salt solution, they are alike in requiring certain changes in the coats or in the embryo, or in both, before germination starts. These changes take place very slowly in dry storage or when the seed is moist at temperatures suitable for germination. Such changes are affected to some extent by different germination conditions, but take place most rapidly in the moist seed at low temperatures at which germination does not occur.

SUMMARY

The progress of germination of seed of the two crabgrasses *Digitaria ischaemum* and *D. sanguinalis* was determined under different conditions of temperature when freshly harvested and after various periods

of storage. The effect of prechilling the moist seed at 3° C. for various periods, as well as the effects of light and of dilute solutions of potassium nitrate, were studied.

Germination was more or less complete at the four temperature alternations used, 20° to 40° C., 20° to 35°, 20° to 30°, and 15° to 25°.

Fresh seed of both species required a preliminary incubation period of 28 to 56 days before germination started; at least 196 days was required for completion of germination.

As the seed aged in dry storage, the preliminary period was shortened and the rate of germination increased. With 1-year-old seed, germination started promptly and was completed in 14 days or less.

Prechilling the fresh seed of both species hastened the rate and the completion of germination. Prechilling for 8 weeks was necessary for prompt germination of fresh seed, but the time required for prechilling decreased as the seed aged.

In a given sample, some seeds were affected by germination treatment differently from others, and the proportion of the seeds stimulated or retarded varied with the treatment.

Freshly harvested seed of *Digitaria ischaemum* had the shortest incubation period at the 20° to 40° C. alternation, but a small proportion of the seeds was slower in germinating at this temperature than at 20° to 35°. At 20° to 30° the incubation period was longer and a larger proportion of the sample was resistant to germination. At 15° to 25° germination started at about the same time as at 20° to 30°, but the rate of germination was slower and with some samples germination was not completed during the period of observation.

Freshly harvested seed of *Digitaria ischaemum* germinated promptly and completely at 20° to 35° C. alternation after 8 weeks' prechilling, but after 2 and 4 weeks' prechilling a portion of the sample was slow to germinate. As the seed aged a shorter period of treatment was sufficient for prompt and complete germination.

Germination of freshly harvested seed of *Digitaria sanguinalis* was essentially the same at 20° to 30° C. and at 20° to 35° alternations, but proceeded at a slower rate at 20° to 40°. At 15° to 25° germination was very slow and incomplete.

The seed of *Digitaria sanguinalis* used was injured by long periods of prechilling, so that maximum germination of fresh seed was not obtained after prechilling. After 1 month of dry storage, prechilling for 4 weeks was followed by fairly prompt and complete germination at 20° to 35° C. alternation; and after 3 or 4 months of dry storage prechilling for 4 weeks resulted in about the same rate of germination as was obtained with 1-year-old seed without chilling.

Treatment of the seed of both species with concentrated sulfuric acid reduced the incubation period of fresh seed, but not as much as an optimum period of prechilling.

Moistening the substratum with 0.2-percent solution of potassium nitrate definitely hastened germination of seed of *Digitaria ischaemum* at the 15° to 25° C. and 20° to 30° alternations, and definitely retarded it at 20° to 35° and 20° to 40°, but had no definite effect on the seed of *D. sanguinalis*.

Total exclusion of light had no definite effect on germination of *Digitaria ischaemum*, but greatly retarded germination of *D. sanguinalis* at 20° to 30° C. alternation and slightly retarded it at 20° to 35°.

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INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA [1]

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INTRODUCTION

For a number of years the United States Department of Agriculture has conducted an extensive lettuce-breeding program. The work has been divided between the United States Horticultural Station at Beltsville, Md., and the United States Horticultural Field Station at La Jolla, Calif.

Since 1935, investigations have been conducted at the Beltsvillle station for the purpose of locating genes for resistance to the aster yellows virus in lettuce. In these tests have been included most of the varieties listed by American seedsmen, as well as many strains received from foreign sources through the Division of Plant Exploration and Introduction. Many varieties of the cultivated species *Lactuca sativa* L. have been grown to determine their resistance or susceptibility to this virus. Since no evidence of resistance was found in any of the varieties tested, an extensive study was made of wild species within the genus *Lactuca*. Some degree of resistance to aster yellows virus has been found in certain of the wild species in tests not yet reported.

For several years workers at the La Jolla station have been studying some of these wild species in breeding for resistance to various diseases and for types better adapted to specific localities.

The question of the genetic compatibility of the various species has naturally arisen. Difficulty in hybridizing some of the wild species with *Lactuca sativa* was expected, owing to the variation in the chromosome number within the genus. According to Babcock, Stebbins, and Jenkins (*1*),[3] the genus *Lactuca* includes species having 8, 9, and 17 pairs of chromosomes.

Little is known of the genetic compatibility of the species of *Lactuca*. Whitaker and Jagger (*9*) have reported on hybridization experiments confined largely to crosses of *L. sativa* with other species. Of nine interspecific crosses attempted, only two, *L. sativa* × *L. Serriola* (*L. scariola*)[4] and *L. graminifolia* × *L. canadensis*, resulted in fertile seed. In both of these the hybrids proved to be fertile.

This paper is a joint report of the results obtained in interspecific hybridization in *Lactuca* at the stations at Beltsville and La Jolla. In the investigations herein reported, numerous interspecific crosses not previously reported were made, with results that cast some light upon the relationship of certain species within the genus.

[1] Received for publication March 18, 1941.

[2] The writers are indebted to the Division of Plant Exploration and Introduction, Bureau of Plant Industry, for supplying much of the species material used in the investigations herein reported. Thanks are extended to G. L. Stebbins, Jr., Division of Genetics, University of California, for material supplied and for identifying some of the species; and also to S. F. Blake, of the Division of Plant Exploration and Introduction, for assistance in identifying some of the species.

[3] Italic numbers in parentheses refer to Literature Cited, p. 107.

[4] The writers have been informed by Dr. S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, that the species generally called *Lactuca scariola* L. should be listed as *L. serriola* L. on the evidence that Linnaeus' first classification used the latter name. This suggested change in the name of the species is supported by Dr. G. L. Stebbins, Jr., of the Department of Genetics, University of California.

MATERIALS AND METHODS

The materials used in the interspecific hybridization studies made by the writers were obtained from the following sources:

Lactuca altaica Fisch. and Mey., from Egypt, through Division of Plant Exploration and Introduction.
L. sativa L., from private stocks.
L. serriola L., collected in the District of Columbia and Maryland.
L. serriola var. *integrata* (Gren. and Godr.) Farwell, collected in the District of Columbia and Maryland.
L. indica L., from China, through G. L. Stebbins, Jr.
L. indica L., from China, through Division of Plant Exploration and Introduction.
L. laciniata (Houtt.) Makino, from Asia, through Division of Plant Exploration and Introduction.
L. tatarica (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.
L. raddeana Maxim., from Union of Socialist Soviet Republics, through Division of Plant Exploration and Introduction.
L. virosa L., from Amsterdam, the Netherlands, through Division of Plant Exploration and Introduction.
L. virosa L., from Surrey, England, through Division of Plant Exploration and Introduction.
L. virosa L., from Stockholm, Sweden, through Division of Plant Exploration and Introduction.
L. canadensis L., collected in Maryland.
L. graminifolia Michx., from South Carolina, through J. B. Norton.
L. floridana (L.) Gaertn., collected in Maryland.
L. saligna L., from Ohio, through G. L. Stebbins, Jr.
L. spicata (Lam.) Hitchc., collected in Maryland, Virginia, and Pennsylvania.
L. perennis L., from Wayside Gardens, Mentor, Ohio.
L. bourgaei (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.
L. cretica Desf., from Africa, through G. L. Stebbins, Jr.
L. chondrillaeflora Bor., from France, through Division of Plant Exploration and Introduction.

In the work at Beltsville, all of the plants used for crossing purposes were grown in 10-inch clay pots in a closely screened greenhouse. At La Jolla the plants were grown outside and the flowers were covered after pollination to prevent contamination.

Some difficulty was met in bringing the different species into flowering at the same time. Some of the species are annual and some biennial or perennial. Species that were found to be easily grown were planted at successive dates so as to have the plants in flower almost continuously.

In making the crosses, the commonly used technique of pollen removal by water, described by Oliver (*6*), was employed.

Diseases and insects caused difficulty in growing the numerous species under the same environment. Some species are very susceptible to disease when grown under glass. *Lactuca canadensis* is susceptible to mildew when grown during the winter months, and several species of the *serriola* group are susceptible to crown rot during cloudy, damp weather. *L. laciniata* is very susceptible to injury by red spider and a number of foliage diseases.

RESULTS

The results of the interspecific cross-pollinations are summarized in table 1.

TABLE 1.—*Summary of data on interspecific hybridization in Lactuca* [1]

Species and number of chromosomes	At Beltsville, Md.				At La Jolla, Calif.			
	Results of cross		Fertility of F_1		Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. sativa (9)×:								
L. altaica (9)	T	T	F	F				
L. serriola (9)	T	T	F	F	T	T	F	F
L. saligna (9)	O	O			N	N		
L. indica (9)	O	O			N	N		
L. tatarica (9)	O	O			O	O		
L. laciniata (9)	O	O			O	O		
L. raddeana (9)	O	O			N	N		
L. virosa (9)	O	O			T		S	
L. perennis (9)	O	N			O	O		
L. bourgaei (8)	O	O			N	N		
L. cretica (8)	O	N			O	O		
L. spicata (17)	O	O			N	N		
L. canadensis (17)	O	O			O	O		
L. graminifolia (17)	O	O			O	O		
L. floridana (17)	O	O			O	O		
L. chondrillaeflora (9)	N	N			O	O		
L. serriola (9)×:								
L. indica (9)	O	N			N	N		
L. tatarica (9)	O	O			N	N		
L. laciniata (9)	O	O			N	N		
L. raddeana (9)	O	N			N	N		
L. perennis (9)	O	N			N	N		
L. virosa (9)	O	O			N	N		
L. bourgaei (8)	O	N			N	N		
L. canadensis (17)	N	O			N	N		
L. graminifolia (17)	O	O			N	N		
L. floridana (17)	O	O			N	N		
L. virosa (9)×:								
L. cretica (8)	O	N			O	N		
L. chondrillaeflora (9)	N	N			O	O		
L. tatarica (9)	O	O			N	N		
L. indica (9)	O	O			N	N		
L. laciniata (9)	O	N			O	O		
L. saligna (9)	O	O			N	N		
L. raddeana (9)	O	O			N	N		
L. perennis (9)	O	O			O	N		
L. graminifolia (17)	O	O			N	T		PF
L. floridana (17)	O	N			N	N		
L. canadensis (17)	O	O			O	O		
L. spicata (17)	O	N			N	N		
L. saligna (9)×:								
L. serriola (9)	T	O	PF		N	N		
L. indica (9)	O	N			N	N		
L. tatarica (9)	O	O			N	N		
L. laciniata (9)	O	N			N	N		
L. raddeana (9)	O	N			N	N		
L. canadensis (17)	O	N			N	N		
L. floridana (17)	O	N			N	N		
L. graminifolia (17)	O	O			N	N		
L. tatarica (9)×:								
L. laciniata (9)	T	T	S	S	N	N		
L. indica (9)	T	T	S	S	N	N		
L. perennis (9)	O	O			N	N		
L. floridana (17)	T	N	S		N	N		
L. muralis (9)	O	O			N	N		
L. raddeana (9)	O	T		S	N	N		
L. laciniata (9)×:								
L. indica (9)	T	T	F	F	N	N		
L. floridana (17)	T	T	S	S	N	N		
L. chondrillaeflora (9)	N	N			O	O		

[1] Key to letter symbols:
F, the hybrids obtained were self-fertile.
N, the cross was not attempted.
O, cross was attempted but failed.
PF, the hybrids were only partly fertile.
S, the hybrids obtained were self-sterile.
T, hybrid seedlings were obtained.
U, hybrids were obtained, but fertility is not yet known.

TABLE 1.—*Summary of data on interspecific hybridization in Lactuca*—Continued

Species and number of chromosomes	At Beltsville, Md.				At La Jolla, Calif.			
	Results of cross		Fertility of F_1		Results of cross		Fertility of F^1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. perennis (9)×:								
L. indica (9)	O	O			N	N		
L. cretica (8)	O	O			N	N		
L. cretica (8)×:								
L. indica (9)	O	N			N	N		
L. raddeana (9)	O	O			N	N		
L. virosa (9)	O	N			N	N		
L. graminifolia (17)	O	O			N	N		
L. raddeana (9) × *L. indica* (9)	T	O	PF		N	N		
L. canadensis (17)×:								
L. tatarica (9)	T	T	S	S	N	N		
L. muralis (9)	O	O			N	N		
L. floridana (17)	T	N	PF		O	O		
L. spicata (17)	T	T	U	U	N	N		
L. raddeana (9)	T	N	S		N	N		
L. graminifolia (17)×:								
L. canadensis (17)	T	T	F	F	T		F	
L. indica (9)	T	N	PF		N	N		
L. laciniata (9)	T	N	S		T	N	S	
L. raddeana (9)	T	N	S		N	N		
L. muralis (9)	T	N	S		N	N		
L. floridana (17)	T	T	PF	PF	O	O		
L. tatarica (9)	T	N	S		N	N		
L. floridana (17)×:								
L. bourgaei (8)	N	N			O	O		
L. indica (9)	T	O	S		N	N		
L. raddeana (9)	T	N	S		N	N		
L. spicata (17)×:								
L. graminifolia (17)	T	N	S		N	N		
L. indica (9)	T	N	PF		N	N		
L. tatarica (9)	T	N	S		N	N		
L. raddeana (9)	T	N	S		N	N		

Of the 81 cross-pollinations reported in this paper, only 29 were successful. Only 10 of the 29 successful crosses produced fertile embryos and 6 of these were partly self-sterile. Sterility of the hybrids in most cases means self-sterility; in only a few cases were the hybrids tested for sterility by backcrossing or outcrossing.

The character of plants obtained from these interspecific crosses varied greatly. In some cases, as in *Lactuca spicata*×*L. graminifolia*, *L. laciniata* × *L. floridana*, *L. graminifolia* × *L. raddeana*, *L. graminifolia* × *L. muralis*, *L. canadensis* × *L. raddeana*, *L. spicata* × *L. tatarica*, *L. floridana* × *L. raddeana*, the hybrids were all weak, abnormal, freakish plants, unable to survive beyond the seedling stage. Some of these weak hybrids had very abnormal leaves, in some cases long and narrow, almost grasslike. In many instances the stem apex appeared to be incapable of growth and elongation. Sometimes the growing point became enlarged.

In some cases the hybrids were all normal except for self-sterility. Where it is stated that the hybrids were strong or vigorous normal plants, reference is made to the general appearance of the plants. The hybrids are indicated as normal if they developed with a fair amount of vigor and showed no tendency to be freakish, even though they were self-sterile.

In a number of instances the hybrids resulting from a cross varied from very weak abnormal plants to strong normal-appearing ones. Such variation was noted among the hybrids from *Lactuca tatarica* × *L. indica*, *L. floridana* × *L. tatarica*, *L. graminifolia* × *L. floridana*, *L. canadensis* × *L. indica*, *L. canadensis* × *L. tatarica*, and *L. indica* × *L. laciniata*. Even though most of the hybrids from the last-named cross were strong normal plants, producing fertile seed, two abnormal freaks appeared in the F_1 population.

CROSSES BETWEEN NINE-CHROMOSOME SPECIES

Lactuca sativa (9) × *L. serriola* (9) (fig. 1, *A*).—This is one of the three successful crosses made with the cultivated species, *L. sativa*. *L. sativa* and *L. serriola* cross readily, and the resulting hybrid produces fertile seed, regardless of which species is used as the maternal parent.

Crosses between these two species have been reported previously by Durst (*2*), Ernst-Schwarzenbach (*3*), Whitaker and Jagger (*9*), and Thompson (*8*). In the present studies *Lactuca sativa* was crossed with both lobed and unlobed forms of *L. serriola*. The F_1 hybrids between these two species are strong vigorous plants in which the characteristics of *L. serriola* predominate.

Lactuca sativa (9) × *L. altaica* (9) (fig. 1, *B*).—Reciprocal crosses were easily obtained between these species. *L. altaica* is a wild type from Egypt. The F_1 plants were vigorous and in many characters resembled the *altaica* parent. The progenies were highly fertile.

Lactuca sativa and *L. altaica* were probably derived from *L. serriola*, since they have the same chromosome number, cross readily, and are similar to *L. serriola* in a great many morphological characters.

Lactuca sativa (9) × *L. virosa* (9) (fig. 1, *C*).—This cross was made by pollinating flowers of *L. sativa* var. Imperial D with pollen of *L. virosa*. Six F_1 plants were produced. Repeated attempts to obtain the reciprocal cross resulted in failure. In most respects the F_1 plants were approximately intermediate between Imperial D and *L. virosa*. They were exceedingly vigorous, some reaching a height of over 12 feet. The annual habit of Imperial D was dominant, and there was some pigmentation in the stem and midrib similar to that found in *L. virosa;* however, there was no pigmentation of the leaf blade proper as is characteristic of *L. virosa*. Like Imperial D, the under surface of the midrib of the leaf was free of spines. All F_1 plants were completely sterile. Attempts to backcross the F_1 hybrids to both parental species resulted in failure. Many attempts at Beltsville to cross these two species both ways failed.

Lactuca tatarica (9) × *L. laciniata* (9) (fig. 1, *D*).—Reciprocal hybrids were obtained between these two species. Two populations of hybrids were grown. The hybrid seedlings in both cases were very weak, the basal leaves having a tendency to turn yellow and fall off, leaving only a few terminal leaves to carry on growth. As the stems elongated, more of the lower leaves turned yellow and dropped. A few plants were grown to a height of 1 to 2 feet and almost to the flowering stage, but in every case the plants died before producing flowers. Many of the characters of the *L. tatarica* parent predominated.

Under greenhouse conditions at Beltsville, *Lactuca tatarica* was completely self-sterile. Under field conditions *L. tatarica* set seed at both Beltsville and La Jolla.

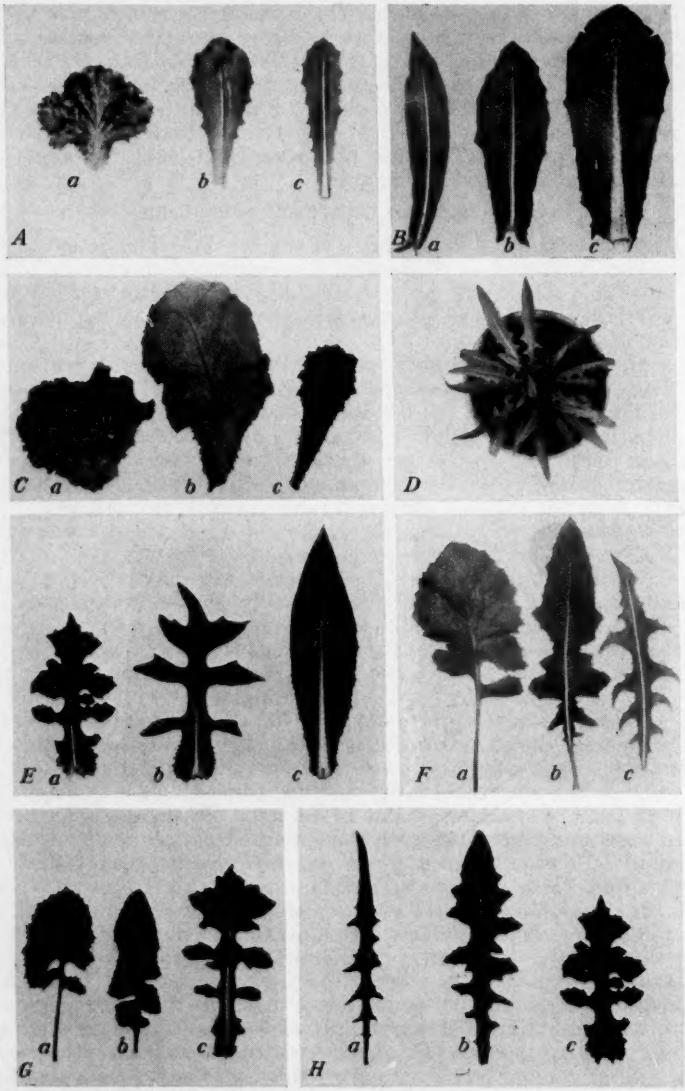

FIGURE 1.—*A*, Stem leaves of (*a*) *Lactuca sativa* var. Mignonette, (*b*) *L. sativa* var. Mignonette × *L. serriola*, F_1, and (*c*) *L. serriola*; *B*, stem leaves of (*a*) *L. altaica*, (*b*) *L. altaica* × *L. sativa* var. Dark Green Cos, F_1, and (*c*) *L. sativa* var. Dark Green Cos; *C*, rosette leaves of (*a*) *L. sativa* var. Imperial D, (*b*) *L. sativa* var. Imperial D × *L. virosa*, F_1, and (*c*) *L. virosa*; *D*, plant of *L. tatarica* × *L. laciniata*, F_1, in the rosette stage; *E*, stem leaves of (*a*) *L. indica*, (*b*) *L. indica* × *L. laciniata*, F_1, and (*c*) *L. laciniata*; *F*, rosette leaves of (*a*) *L. raddeana*, (*b*) *L. raddeana* × *L. tatarica*, F_1, and (*c*) *L. tatarica*; *G*, rosette leaves of (*a*) *L. raddeana*, (*b*) *L. raddeana* × *L. indica*, F_1, and (*c*) *L. indica*; *H*, stem leaves of (*a*) *L. tatarica*, (*b*) *L. tatarica* × *L. indica*, F_1 and (*c*) *L. indica*.

Lactuca indica (9) × *L. laciniata* (9) (fig. 1, *E*).—Reciprocal crosses between these species were successful; however, the cross is more easily made by using *L. laciniata* as the maternal parent. The flowers of *L. indica* are easily injured in handling while removing the pollen; hence it is best used as the pollen parent. Two weak, abnormal plants appeared among the hybrids.

The normal F_1 plants from *Lactuca indica* × *L. laciniata* were larger and more vigorous than either parent. The F_1 plants have many of the characters of *L. indica* and look much like this parent. The hybrids were highly fertile and produced abundant seed.

The F_1 hybrid was backcrossed with each of the original parents. Backcrosses with the parents were obtained by pollinating *Lactuca laciniata* with hybrid pollen and by pollinating flowers of the hybrid with pollen from *L. indica*. In both cases the progenies were fertile.

Merrill (*4*) considers *Lactuca indica* and *L. laciniata* to be synonymous. The results obtained from crossing them indicate that *L. laciniata* should be classed as a variety of *L. indica*.

Lactuca raddeana (9) × *L. tatarica* (9) (fig. 1, *F*).—Ten hybrid plants resulted from numerous crosses made between these two species. No hybrids were obtained when *L. tatarica* was used as the maternal parent. The *L. tatarica* plant used in the cross was old and in a very weak condition at the time the crosses were made. This may account for the failure to obtain hybrids when *L. tatarica* was used as the mother plant. Of the 10 hybrids, 8 were weak deformed plants and died while small. The normal hybrids were vigorous and larger than either parent. In the rosette stage they appeared to be intermediate between the parents. The hybrids had a marked tendency to stool and produced numerous stems arising from the crown. The hybrids were all self-sterile.

Lactuca raddeana (9)×*L. indica* (9) (fig. 1, *G*).—From a small population of plants grown from seed obtained by crossing *L. indica* on *L. raddeana*, two hybrids were obtained. Attempts to obtain the reciprocal cross failed. In general appearance the hybrids more nearly resembled *L. indica* than *L. raddeana*, but were less leafy and had longer internodes. The leaves had the dark-green color of *L. indica*. Neither hybrid plant showed any tendency to branch just above the crown as did the *L. raddeana* parent. The heads were intermediate in number of flowers per head. *L. raddeana* is biennial or perennial, and *L. indica* is an annual. The hybrid flowered without overwintering.

The hybrids were at first thought to be completely self-sterile, but a few heads on each plant set seed late as the plants were on the verge of dying. It was found necessary to harvest the seeds a few days before they were mature and to germinate them immediately. If the seeds were permitted to ripen completely on the plant the embryos shriveled and died. No progeny was obtained from mature seed. A few plants are now being grown from seed harvested prematurely.

Lactuca tatarica (9)×*L. indica* (9) (fig. 1, *H*).—Seed set very readily in flower heads of *L. tatarica* to which pollen of *L. indica* was applied. The reciprocal cross was also obtained. Several populations of hybrid plants have been grown from crosses between these two nine-chromosome species. A large number of the hybrids have been grown to maturity, but all were completely sterile. In each population of

hybrids there were a number of very weak malformed plants. Some of these were grown in pots for several months, but in every case they died without producing a seed stem.

In general appearance the hybrids resembled *Lactuca tatarica* much more than *L. indica*. The foliage had the gray-green color of *L. tatarica*, although the flower heads were a little smaller and the corollas a lighter blue.

CROSSES BETWEEN 17-CHROMOSOME SPECIES

Lactuca canadensis (17)×*L. floridana* (17) (fig. 2, *A*).—Flowers of *L. canadensis* were pollinated with pollen from *L. floridana*. From the seed obtained 15 plants were grown, 3 of which were weak and very deformed. Although kept in pots for several months, they failed to develop. The remaining seedlings were strong and vigorous.

Although both the species used are normally biennial, two of the plants developed seed stems the first season and both proved to be hybrids. Both plants were strong and vigorous, but they were no taller and no heavier stemmed than *L. canadensis* and indicated little hybrid vigor. In general appearance they were intermediate between the two parents. The flowers were intermediate in size, and the corollas were blue of a little lighter shade than those of *L. floridana*.

Both hybrid plants were partially self-sterile, although both produced a few seeds. An F_2 population is being grown.

Lactuca graminifolia (17) × *L. canadensis* (17) (fig. 2, *B* and *C*).—This cross can be readily made in both directions. The F_1 plants are intermediate between the two species in most respects, including height, size and shape of leaf, color and shape of ligules, and color and size of achenes. The writers' observations indicate that the annual habit and the gray color of the pollen grains of *L. graminifolia* are dominant over the biennial habit and the orange-colored pollen of *L. canadensis*. The F_1 was as fertile as either parent.

Lactuca canadensis (17) × *L. spicata* (17) (fig. 2, *D*).—From a population of 25 plants grown from seed resulting from this cross, with *L. spicata* as the pollen parent, 20 hybrids were obtained. In the rosette stage the hybrids resembled *L. spicata* more than *L. canadensis*. The fertility of the hybrids is not known, since none have reached the flowering stage. The reciprocal cross was easily obtained.

Lactuca morssii Robinson, as described in Gray's Manual (*7*), is generally considered to be a hybrid between *L. spicata* and *L. canadensis*. This form is found at low elevations in parts of Maine and Massachusetts. Wiegand and Eames (*10*) report natural hybrids between these two species occurring in the wild in New York State.

Lactuca spicata (17) × *L. graminifolia* (17).—Eighteen hybrid plants were obtained from this cross, *L. graminifolia* being used as the pollen parent. All of the hybrids were abnormal, weak plants unable to survive beyond the seedling stage. The reciprocal cross failed.

Lactuca graminifolia (17) × *L. floridana* (17) (fig. 2, *E*).—Reciprocal crosses were obtained between these two species. All of the 17 hybrids produced by using *L. graminifolia* pollen were weak, abnormal plants. Of the 22 hybrids obtained by using *L. floridana* as the pollen parent, 11 were abnormal and freakish and 11 were normal. The plants that flowered were partially fertile.

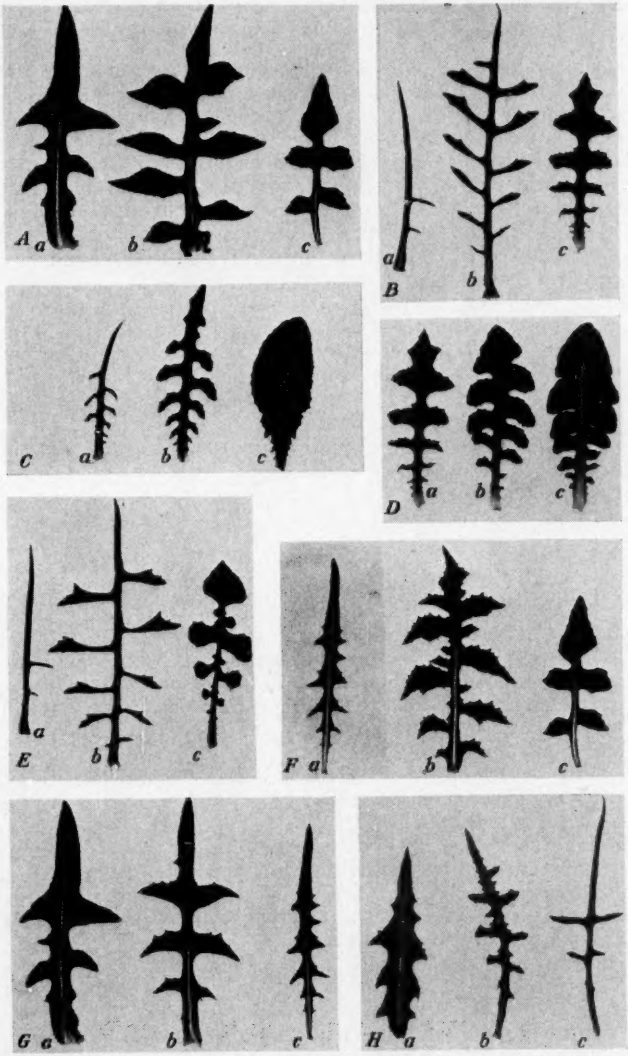

FIGURE 2.—*A*, Stem leaves of (*a*) *Lactuca canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. floridana*, F_1, and (*c*) *L. floridana*; *B*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. canadensis* var. *latifolia*, F_1, and (*c*) *L. canadensis* var. *latifolia*; *C*, rosette leaves of (*a*) *L. graminifolia*, (*b*), *L. graminifolia* × *L. canadensis* var. *integrifolia*, F_1, and (*c*) *L. canadensis* var. *integrifolia*; *D*, rosette leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. spicata*, F_1, and (*c*) *L. spicata*; *E*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. floridana*, F_1, and (*c*) *L. floridana*; *F*, stem leaves of (*a*) *L. tatarica*, (*b*) *L. tatarica* × *L. floridana*, F_1, and (*c*) *L. floridana*; *G*, stem leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. tatarica*, F_1, and (*c*) *L. tatarica*; *H*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. tatarica*, F_1, and (*c*) *L. tatarica*.

CROSSES BETWEEN 9- AND 17-CHROMOSOME SPECIES

Lactuca tatarica (9) × *L. floridana* (17) (fig. 2, *F*).—*L. tatarica*, an annual species having 9 pairs of chromosomes, was used as the maternal parent in a cross with the biennial species *L. floridana*, having 17 pairs of chromosomes. Both of these species are blue-flowered. The flowers of *L. tatarica* are much larger than those of *L. floridana* and the anther sheath is less pigmented. Both have pinnately lobed leaves.

Eight hybrid plants were grown from the seed obtained from the cross-pollinations. All were self-sterile and behaved as annuals. As in most of the cases of self-sterility observed in *Lactuca*, these plants continued to grow and produced flowers over a long period.

The hybrid plants showed marked hybrid vigor in the rosette stage but did not develop extremely large seed stems. The flowers were blue and about the same size as those of *Lactuca tatarica*. The dark pigmentation of the anther sheaths in *L. floridana* was dominant. The leaves were a gray green similar to those of *L. tatarica*, but they were much larger and broader than in either parent. The annual habit of *L. tatarica* was dominant.

Cytological examination of root-tip material showed the somatic cells of the hybrids to have 26 pairs of chromosomes.

Lactuca canadensis (17) × *L. tatarica* (9) (fig. 2, *G*).—Several flower heads of the 17-chromosome species *Lactuca canadensis* were cross-pollinated with the 9-chromosome species *L. tatarica*. Of the plants grown from these cross-pollinations, 34 proved to be interspecific hybrids having 26 pairs of chromosomes. All hybrids were completely self-sterile. The plants were quite uniform in general appearance and resembled the *L. tatarica* parent in many characteristics. The parent plants apparently were somewhat heterozygous. Although the hybrids were uniform in general appearance, close examination indicated considerable variation. Of the 34 hybrids, 5 were small and weak as compared with the average of the population, and 3 of these died before reaching the flowering stage. All the hybrids carried anthocyanin pigment in their leaves but were not identical in type of pigmentation; 18 were of the tinged type [5] and 16 were more heavily pigmented, approaching the red type. The green of the leaves approached the gray green of the *L. tatarica* parent. Some of the hybrids developed a thick brown epidermis that had a tendency to crack as the plants reached maturity. Counts on the 16 mature plants showed 10 to have this character in some degree. All the plants having this thick outer layer appeared deformed.

The flowers were intermediate in size. The color of the corolla was nearly the same shade of blue as that of *Lactuca tatarica*, showing dominance of blue over the yellow color characteristic of *L. canadensis*. These plants produced an enormous number of flowers over a long period, but no seed developed.

Sprouting from the roots, a characteristic of *Lactuca tatarica*, appears to be dominant. The seed stems were removed from 16 plants after they had been in flower for several weeks. Most of them had developed sprouts from the roots 2 months after the seed stem was removed. The hybrids did not show a tendency to sprout or stool from the crown as *L. tatarica* does. The annual habit was dominant. Reciprocal hybrids were obtained, but none were grown to maturity.

[5] The leaf-color types were based on the classification by Thompson (*8*).

Lactuca graminifolia (17) × *L. tatarica* (9) (fig. 2, *H*).—The third cross between a 17-chromosome species and the 9-chromosome species *Lactuca tatarica* was obtained by applying pollen from *L. tatarica* to flowers of *L. graminifolia*. Fifteen hybrid plants were grown from this cross, all of which were sterile. As in the crosses of *L. tatarica* with *L. canadensis* and *L. floridana*, the hybrids more nearly resembled the *L. tatarica* parent. The gray green of *L. tatarica* also appeared to be dominant in this case. The plants were a little larger and more vigorous than either parent.

The flowers were intermediate in size and of about the same shade of blue as those of the *Lactuca tatarica* parent. Some of the hybrids sprouted or stooled at the crown like *L. tatarica*, while others produced only a single stem characteristic of *L. graminifolia*. Like both parent species, the hybrids behaved as annuals, producing flowers the first season. However, there was a great difference in time of seed-stem elongation among the hybrids, nine of them producing seed stems much earlier than the remaining six. The individual plants within the two groups elongated at about the same time and rate. Plants that stooled were slower to develop seed stems than single-stem plants.

Lactuca laciniata (9) × *L. floridana* (17).—Eighty-three hybrid plants were grown from seed obtained by applying pollen of *Lactuca floridana* to flowers of *L. laciniata*, and 11 were obtained from the reciprocal cross. The hybrids were all very weak and unable to develop beyond the seedling stage, regardless of which way the cross was made. The hybrid seedlings developed five to six leaves, then turned yellow and died. These plants were readily distinguished from either parent, and there was no doubt of their being hybrids. Progenies from this cross were planted at three different dates, but in each case the hybrids were weak and died after developing a few leaves.

Lactuca floridana (17) × *L. indica* (9) (fig. 3, *A*).—Several flower heads of *Lactuca floridana* were crossed with *L. indica*. Only one hybrid plant was obtained from the seed from this cross. The plant was intermediate in size and resembled the *L. indica* parent more than *L. floridana*. It had anthocyanin spots on the leaves like *L. indica*, but they were much larger and of a more intense red. The flowers were intermediate in size and the corollas blue, of about the shade of *L. floridana*. The reciprocal cross failed.

Lactuca floridana generally behaves as a biennial, and *L. indica* is annual in habit. The hybrid plant was an annual and completely sterile.

Lactuca graminifolia (17) × *L. raddeana* (9).—Fifty-three plants were grown from seed resulting from the application of pollen of *Lactuca raddeana* to flowers of *L. graminifolia*. Of these, 48 were hybrids. The hybrid plants were all very weak and died in the seedling stage.

Lactuca graminifolia (17) × *L. muralis* (9).—Pollen of *L. muralis* applied to flowers of *L. graminifolia* resulted in 9 hybrids out of a population of 14 plants. All the hybrids were weak and unable to survive beyond the seedling stage. This is probably the widest cross obtained so far, for the two species differ greatly in many morphological characters.

Lactuca canadensis (17) × *L. raddeana* (9).—A population of 23 plants was grown from seed obtained by applying pollen of *L. raddeana*

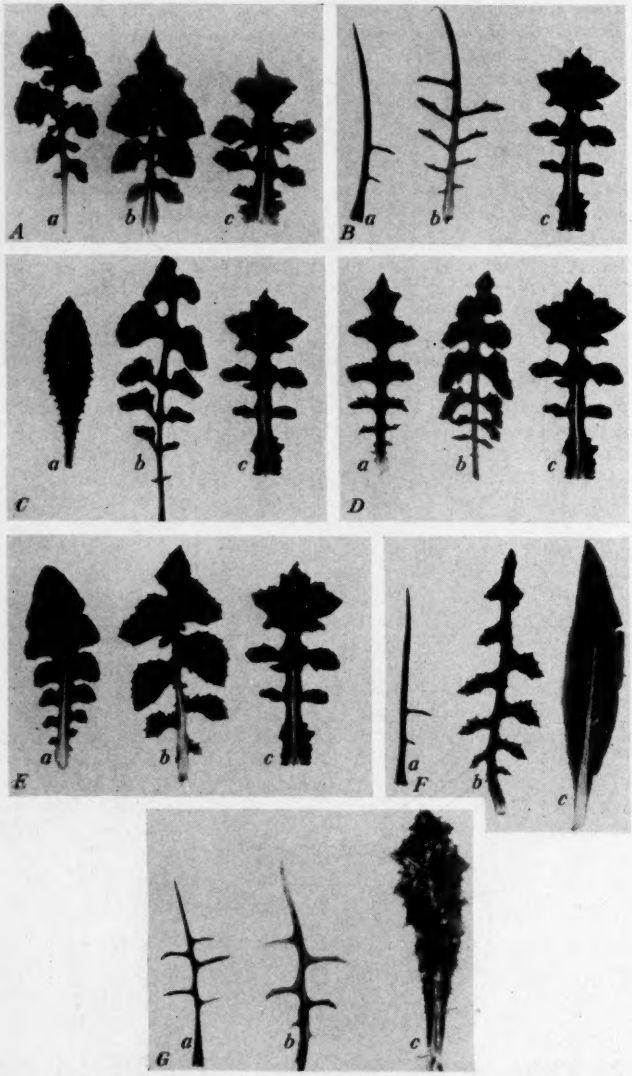

FIGURE 3.—*A*, Stem leaves of (*a*) *Lactuca floridana*, (*b*) *L. floridana* × *L. indica*, F_1, and (*c*) *L. indica*; *B*, stem leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *C*, rosette leaves of (*a*) *L. canadensis* var. *integrifolia*, (*b*) *L. canadensis* var. *integrifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *D*, rosette leaves of (*a*) *L. canadensis* var. *latifolia*, (*b*) *L. canadensis* var. *latifolia* × *L. indica*, F_1, and (*c*) *L. indica*; *E*, rosette leaves of (*a*) *L. spicata*, (*b*) *L. spicata* × *L. indica*, F_1, and (*c*) *L. indica*; *F*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. laciniata*, F_1, and (*c*) *L. laciniata*; *G*, rosette leaves of (*a*) *L. graminifolia*, (*b*) *L. graminifolia* × *L. virosa*, F_1, and (*c*) *L. virosa*.

to flower heads of *L. canadensis*. Of the 23 plants 10 were very weak hybrids and all of these died in the seedling stage. Normal hybrids were not obtained.

Lactuca graminifolia (17) × *L. indica* (9) (fig. 3, *B*).—From seed obtained by applying pollen of *L. indica* to flowers of *L. graminifolia*, 29 hybrids were obtained. All were normal, vigorous plants, producing small, dark reddish-blue flowers similar to those of *L. graminifolia*. Some of these hybrids produced flowers and a few seeds, indicating partial fertility.

Lactuca spicata (17) × *L. tatarica* (9).—Four hybrids obtained from this cross with *L. tatarica* as the pollen parent were all weak, abnormal plants and were unable to survive beyond the seedling stage.

Lactuca canadensis (17) × *L. indica* (9) (fig. 3, *C* and *D*).—All but 5 of 19 hybrids from this cross with *L. indica* as the pollen parent were weak, abnormal plants and died in the seedling stage. Some of the normal hybrid plants have come to the flowering stage and are self-sterile.

Lactuca spicata (17) × *L. raddeana* (9).—Five hybrid plants were obtained from seed resulting from the application of *L. raddeana* pollen to flowers of *L. spicata*. All were weak and died in the seedling stage.

Lactuca spicata (17) × *L. indica* (9) (fig. 3, *E*).—Fifteen hybrid plants were obtained from a cross between *L. spicata* and *L. indica* in which the latter was used as the pollen parent. The hybrids were strong, vigorous plants. In the seedling stage they looked more like *L. indica* than *L. spicata*. The leaves were more lobed than those of *L. spicata* and had large spots of dark-red anthocyanin. Some of the hybrids have reached the flowering stage and one has produced a few seeds.

Lactuca floridana (17) × *L. raddeana* (9).—Sixteen hybrid plants were obtained from this cross, *L. raddeana* being used as the pollen parent. All the hybrids were weak, abnormal plants and died in the seedling stage.

Lactuca graminifolia (17) × *L. laciniata* (9) (fig. 3, *F*).—Seven hybrid plants were obtained from this mating at La Jolla, Calif. All of them had considerable dark-red pigment along the midrib of the leaf, similar to that on *L. laciniata*. These plants were unmistakable hybrids and were very weak. They were susceptible to a variety of foliage diseases, and none developed past the rosette stage. Twenty-two hybrids were obtained at Beltsville, Md., all but one of which were weak and died in the seedling stage.

Lactuca graminifolia (17) × *L. virosa* (9) (fig. 3, *G*).—One F_1 plant was obtained by pollinating flowers of *Lactuca graminifolia* with *L. virosa* pollen. This plant was similar to *L. graminifolia* in most respects. It was an annual and of about the same height and shape as *L. graminifolia*. It was positively identified as a hybrid by the fact that the leaf blades were characterized by numerous small patches of pigment on the upper surface of the leaf blade, like *L. virosa*. The flowers had the purple-colored ligules of *L. graminifolia*, but the purple was much lighter in the hybrid.

DISCUSSION

The results obtained from the interspecific crosses reported above give some interesting information regarding the genetic relationship of certain species within the genus *Lactuca*.

The breeding behavior of the 18 forms studied indicates that, excepting the 8-chromosome species *Lactuca bourgaei* and *L. cretica* and the 9-chromosome species *L. muralis*, *L. perennis*, and *L. chondrillaeflora*, all belong to three quite distinct compatibility groups: (1) The group hereinafter called the *serriola* group, consisting of *L. serriola*, *L. sativa*, *L. saligna*, *L. altaica*, and *L. virosa*; (2) the group hereinafter called the *indica* group, consisting of *L. indica*, *L. laciniata*, *L. raddeana*, and *L. tatarica*; and (3) the group consisting of the four 17-chromosome species *L. canadensis*, *L. spicata*, *L. floridana*, and *L. graminifolia*.

In group 1, *Lactuca sativa*, *L. serriola*, and *L. altaica* are indicated to be varieties of the same species, since they cross readily in either direction, with fertile progenies, and have many morphological characters in common. In group 2, *L. laciniata* is indicated to be a variety of *L. indica*, since the two forms have many morphological similarities and cross readily, giving fertile progenies.

Though hundreds of cross-pollinations were made between species in group 1 and those in group 2, not a single hybrid was obtained, suggesting a remote genetic relationship between these two groups of 9-chromosome species.

Although *Lactuca indica* and *L. laciniata* are morphologically closely related to *L. serriola*, *L. saligna*, and *L. virosa*, and are taxonomically placed in the subgenus *Serriola*, there is evidence of some genetic relationship with *L. raddeana* and *L. tatarica* in the subgenus *Mulgedium*.

The four species with 17 chromosomes have been separated by taxonomists into the two subgenera *Serriola* and *Mulgedium* largely on the characters of the achenes and involucres. *Lactuca canadensis* and *L. graminifolia* have been assigned to the subgenus *Serriola* and *L. floridana* and *L. spicata* to the subgenus *Mulgedium*. Four crosses were obtained between these two subgenera, namely, *L. canadensis* × *L. floridana*, *L. canadensis* × *L. spicata*, *L. graminifolia* × *L. floridana*, and *L. spicata* × *L. graminifolia*. The first-named cross gave some fertile progenies. Fertile hybrids of the second cross, *L. canadensis* × *L. spicata*, have very likely developed in nature (*7*, *10*). The third cross, *L. graminifolia* × *L. floridana*, gave partially fertile progenies. The fourth cross, *L. spicata* × *L. graminifolia*, is known to produce self-sterile progenies. A fifth cross between two 17-chromosome species in the subgenus *Serriola* (*L. graminifolia* × *L. canadensis*) gave fertile progenies. These results indicate a close genetic relationship among all four of these species.

Sixteen successful crosses were made between 17- and 9-chromosome species. All of these except one involved 9-chromosome species within the *indica* group. The only hybrid plant obtained between a 17-chromosome species and the *serriola* group was at La Jolla, Calif., between *Lactuca graminifolia* and *L. virosa*, when *virosa* pollen was used. Table 1 shows the many attempted crosses between the 17-chromosome group and the 9-chromosome *serriola* group. In most cases at least 10 flower heads were cross-pollinated, and in

many cases a large number of crosses were tried. Most of these attempts were made under conditions favorable for fertilization, yet only the one hybrid plant was obtained.

The ease with which crosses were obtained between the 17-chromosome species and the 9-chromosome *indica* group, as compared with the almost total failure to obtain crosses between the 17-chromosome group and the *serriola* group, indicates a much closer genetic relationship between the 17-chromosome group and the *indica* group than between the 17-chromosome group and the *serriola* group. This is closely paralleled by the similarity between the two groups in gross morphology.

It is generally assumed that the 17-chromosome species, *Lactuca canadensis*, *L. spicata*, *L. floridana*, and *L. graminifolia*, are amphidiploids, having arisen from hybrids between 8- and 9-chromosome species. If this assumption is correct, the behavior of the interspecific hybrids herein reported indicates that the 9-chromosome species contributing to the amphidiploids were more likely to have been members of the *indica* group or closely related species than of the *serriola* group.

The sterility of many of the interspecific hybrids prevents any extensive study of the inheritance of characters; however, an apparent dominance of certain characters was noted in the F_1 populations. The annual is dominant over the biennial habit, as indicated by the matings of *Lactuca canadensis* × *L. tatarica*, *L. floridana* × *L. tatarica*, and *L. floridana* × *L. indica*. *L. canadensis* and *L. floridana* are both biennials—at least the stocks used in these studies were biennials; *L. indica* and *L. tatarica* are annuals. All of the hybrids were annuals.

Where lobed-leaved species were crossed with lanceolate nonlobed species, the hybrids were always lobed, indicating dominance of this character.

Light-colored, gray, or whitish color of pollen was dominant over orange in *Lactuca graminifolia* × *L. canadensis*, *L. canadensis* × *L. tatarica*, and *L. graminifolia* × *L. indica* crosses, in which *L. graminifolia* and *L. tatarica* have light-colored pollen.

The gray-green foliage color of *Lactuca laciniata* was dominant over other foliage greens in *L. laciniata* × *L. indica* and *L. floridana* × *L. laciniata*.

The tendency to sprout from the roots, characteristic of *Lactuca tatarica*, appeared to be a dominant character in the hybrids *L. canadensis* × *L. tatarica*, *L. floridana* × *L. tatarica*, *L. tatarica* × *L. indica*, and *L. graminifolia* × *L. tatarica*.

The susceptibility of *Lactuca laciniata* to certain foliage diseases appeared to be dominant, as most of the hybrids in which this species was a parent were subject to similar maladies, which added to the difficulties of growing the hybrids to the flowering stage.

Anthocyanin pigment in the foliage, stems, and flowers was in every case dominant over its absence.

The membranous margins on the achenes of *Lactuca canadensis* were dominant in *L. canadensis* × *L. tatarica* and *L. canadensis* × *L. floridana* crosses. Although *L. canadensis* × *L. tatarica* hybrids were sterile, examination of the infertile ovaries showed them to have this type of margin.

Few weak spines, or no spines, on the under side of the midrib of the leaf seem to be dominant over many coarse spines.

In most crosses between 9- and 17-chromosome species, hybrids were obtained only when the 9-chromosome species was used as the pollen parent. In fact, it is generally considered difficult to effect crosses between species of different chromosome number when the species having the larger number is used as the pollen parent. Müntzing (*5*) discusses at some length this question of crosses between species being more successful when the one having the larger chromosome number is used as the maternal parent, and he offers double fertilization as the possible explanation. In two cases herein reported two species of different chromosome number were crossed in both directions, with hybrids resulting. These were the *Lactuca floridana* × *L. laciniata* and *L. canadensis* × *L. tatarica* crosses.

It is not assumed that these investigations reveal all the possible relationships that exist among the species studied. No doubt, under more nearly optimum conditions for certain of these species, crosses might be effected that are herein reported to have failed. Fertile embryos under certain conditions might develop in hybrids here reported to be completely self-sterile. The hybrid *Lactuca raddeana* × *L. indica* is an example; if the two plants had not been observed carefully in the very last stages of maturity and the immature embryos tested for viability, these hybrids would have been reported as completely self-sterile. Hundreds of crosses were made between *L. virosa* and other members of this nine-chromosome group before hybrids were obtained with *L. serriola*.

The extent of these investigations and the results obtained would seem to establish the close relationship among the species within the two nine-chromosome groups and the more remote relationship, if not complete incompatibility, of the two groups.

These studies have contributed some tangible support to the assumed amphidiploid nature of the 17-chromosome species and give substantial evidence that the 9-chromosome species involved in the synthesis of the amphidiploids was a member or were members of the *indica* group or closely related species. Similarly, the 9-chromosome species included in the *serriola* group are practically eliminated as possible contributors to the synthesis of the amphidiploids.

The results of these studies of interspecific hybridization have one discouraging aspect from the viewpoint of the lettuce breeder; that is, the apparent genetic incompatibility of the cultivated form *Lactuca sativa* with many of the other species of the genus. Hybridization of *L. sativa* appears to be possible only with *L. serriola* and possibly with some very closely related forms. Some of the most promising material for breeding for resistance to certain diseases is in species too remotely related genetically to *L. sativa* to permit hybridization that will result in fertile progenies.

SUMMARY

Results from 81 attempted interspecific crosses among 18 species of the genus *Lactuca* are presented. These crosses involved two 8-chromosome, twelve 9-chromosome, and four 17-chromosome species.

Of the 81 attempted crosses, 29 were successful in producing viable embryos and hybrid seedlings.

Eleven of the successful crosses were self-fertile or partly so. The remaining 18 produced either weak, abnormal seedlings unable to

survive beyond the infantile or rosette stage, or strong, vigorous plants that developed more or less normally but were completely self-sterile.

The four 9-chromosome species *Lactuca indica*, *L. laciniata*, *L. tatarica*, and *L. raddeana* were found to be closely related genetically and capable of crossing among themselves. Except in the case of *L. indica* × *L. laciniata* and *L. raddeana* × *L. indica*, the hybrids were self-sterile.

The nine-chromosome species *L. sativa*, *L. serriola*, *L. altaica*, *L. saligna*, and *L. virosa* were found by breeding behavior to be closely related and capable of crossing among themselves. Some of the hybrids resulting from cross-pollination within this group were as fertile as either parent; others were completely self-sterile.

Although many crosses were attempted between species from the two groups of nine-chromosome species listed above, no hybrids were obtained.

The four 17-chromosome species studied were found to be closely related genetically and capable of crossing among themselves. Some of these crosses resulted in fertile or partially fertile hybrids.

Sixteen attempted crosses between 17- and 9-chromosome species were successful in producing fertilized embryos. All but 2 of these 16 crosses involved a 9-chromosome species in the *indica* group. Crosses between the 17-chromosome species and the 9-chromosome species of the *serriola* group failed in every case but 1, that of *Lactuca graminifolia* × *L. virosa*.

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SULFUR AND TRACE-ELEMENT NUTRITION OF ASPERGILLUS NIGER [1]

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INTRODUCTION

Though it is known that sulfate sulfur disappears in the green plant to reappear as disulfide (cystine) and sulfhydryl (cysteine), little is known concerning this process of reduction, or of the intermediate compounds involved. Nightingale et al. (*12*) [3] found that sulfate is reduced to sulfite and apparently to sulfhydryl in the comparatively alkaline phloem region of roots and tops. Neither Heiserich (*5*) nor Mothes (*10*) was able to bring about reduction of sulfate with macerated green tissue. However, reduction of sulfate to disulfide was reported by Hammett and Reynolds (*4*) to take place with extracts of *Phaseolus vulgaris* root tips. They considered the process to be enzymatic, since the heated extracts did not cause a diminution in sulfate.

According to summaries by Pfeffer (*13*), Jost (*6*), and Miller (*9*), green plants require sulfate and are unable to assimilate any of its reduction products. Recently, however, increased yields have been found to occur with atmospheric sulfur dioxide (0.1 to 0.2 p.p.m.) by Setterstrom (*14*). Though the possibility exists that oxidation of sulfur dioxide occurred prior to use, the experimental data would indicate the desirability of a restudy of sulfur utilization in green plants. There can be little doubt of the presence in plants of sulfur compounds intermediate in oxidation between sulfhydryl and sulfate (*10, 12*). It appears improbable that sulfur is introduced into organic compounds as sulfate by the green plant without previous reduction.

The situation is quite different with respect to fungi. These have been grown successfully with a large number of sulfur compounds (*2, 7, 18, 19*). Here, again, insufficient evidence is available to determine the course of sulfur assimilation except in a general way. Mothes (*10*) was able to determine, however, that the sulfur metabolism of *Aspergillus niger* parallels that of green plants, whereas it diverges from that of the yeasts. Fuller information, in his opinion, may reveal that specific differences in sulfur metabolism also exist between green plants and fungi.

A general survey of the relation between constitution and assimilability of sulfur compounds was therefore undertaken with this fungus.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 126.

A fuller knowledge of sulfur metabolism in *Aspergillus niger* should prove of value in working out that of the green plant. The absence of rapid and sensitive methods for the chemical identification of suspected intermediary forms of sulfur made necessary the adoption of comparisons upon an exactly quantitative basis. This was accomplished by measuring assimilability of sulfur by the yield obtained with 25 mg. of sulfur per liter at 35° C., after 4 days of growth in an optimum solution. The maximum dry weight (1,100 to 1,300 mg.) obtained with sulfate sulfur served as a standard of comparison.

The sulfur compounds studied fall into five groups of which the known reduction products of sulfuric acid are the most important. Other sources of inorganic sulfur are the thionic acids. The naturally occurring sulfur compounds, such as cystine and methionine and their derivatives, fall into a special category. Lastly, there are the miscellaneous organic sulfur compounds, some of natural occurrence, including the oxidation-reduction series of mercaptan sulfonate and of sulfide-sulfone. These are treated separately. These substances are, on the whole, the simple sulfur-containing compounds into which the more complex forms would be transformed before assimilation under ordinary conditions.

The general assumption underlying the experimental procedures and interpretation of data is that the initial stages of sulfur assimilation are essentially a process of chemical reduction (digestion). Armstrong (*2*) and others have found *Aspergillus niger* to be capable of carrying this reduction to the production of elemental sulfur. Inability of the fungus to grow when supplied with sulfur in a reduced form was assumed to imply, in addition, that oxidation of nutritive sulfur is a matter of difficulty for the fungus.

Paralleling the studies on sulfur assimilation were others with the trace elements iron, zinc, copper, manganese, molybdenum, and gallium. The effects of a deficiency of each of these elements were studied with a variety of sulfur compounds in an attempt to determine whether a specific role was played by one of these elements in the (enzymatic) reduction of sulfur compounds. A relationship of this sort has been found between nitrate reduction and molybdenum (*17*).

MATERIAL AND METHODS

Aspergillus niger Van Tiegh. (No. 215–4247 in the collection of Dr. Charles Thom) was grown on 50-cc. portions of a nutrient solution in 200-cc. Erlenmeyer flasks at 35° C. for 4 days. The nutrient solution consisted of redistilled water 1,000 cc., sucrose 50 gm., reagent ammonium nitrate 2.06 gm., reagent dipotassium phosphate 0.35 gm., and reagent magnesium sulfate ($7H_2O$) 0.25 gm. This solution contained 25 mg. of sulfur per liter, or 1.25 mg. of sulfur per culture. Practically spectroscopically pure iron, zinc, copper, manganese, molybdenum, and gallium were supplied as chlorides in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively. The sucrose contained 0.00087 percent of ash. All cultural vessels and accessory apparatus were of transparent quartz, except only the boiling flask of the still and the storage bottle for redistilled water, which were of silica ware. Inoculation was by means of a spore suspension.

When nutrient-solution purification (*15*) was employed, the ammonium nitrate was increased to 2.60 gm. per liter, the dipotassium

phosphate to 0.70 gm., and the magnesium sulfate to 1.10 gm. in the above formula. After the addition of 1 gm. of calcium carbonate and heating to 100° C. for 20 minutes (steamer), the solution was filtered through a fritted quartz crucible of No. 4 porosity. Trace elements were added subsequently.

In solutions provided with other than sulfate-sulfur, magnesium was supplied as $MgCl_2.6H_2O$, 0.21 gm. per liter, or 0.91 gm. per liter when the solution was purified with calcium carbonate. Sulfur compounds other than sulfate were also supplied in concentrations sufficient to provide 25 mg. of sulfur per liter, or, if subjected to nutrient-solution purification, 50 mg. of sulfur per liter

In performing the starch tests a drop of N/20 iodine solution was placed on the reverse of the mycelial felts during filtration, washed several times with water, and examined for starch at ×10 magnification after the lapse of an hour or more. A quinhydrone electrode was used to determine acidity. Yields are given per culture of 50 cc., the mycelial felts having been dried at 103° C. overnight after filtration with fritted glass crucibles of No. 3 porosity.

The sulfur compounds used were reagent chemicals or the purest available.

ASSIMILABILITY OF INORGANIC SULFUR

The maximum yields obtained with sulfate and its products of reduction are tabulated in table 1. It can be seen that as sulfate was successively reduced to sulfite, hyposulfite, sulfoxylate, and sulfide, no diminution in assimilation occurred until the sulfide was formed. Sulfoxylate, though unobtainable, is included here because sodium hyposulfite hydrolyzes in slightly acid solution to form an equimolecular mixture of sulfoxylic and sulfurous acids, which is assimilated as readily as sulfurous acid alone. Utilization of sodium hyposulfite is therefore considered to depend wholly on the formation of sulfoxylate. The bisulfite residue is presumably reduced again to sodium hyposulfite by the fungus. It does not seem probable from the chemical data as summarized by Mellor (*8*) that sulfoxylate has been produced directly by reduction of hyposulfite, though sulfide can be so formed. It is clear, at any rate, that sodium sulfoxylate should also be capable of giving maximum yields, and that it is the lowest state of oxidation in which inorganic sulfur can be utilized efficiently.

TABLE 1.—*Growth data for Aspergillus niger after 4 days at 35° C. with inorganic compounds providing 25 mg. of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. sucrose	Sporulation [1]
		Mg.	
Potassium persulfate	$K_2S_2O_8$	716.2+	2
Sodium sulfate	Na_2SO_4	1,173.0	8
Sodium bisulfite	$NaHSO_3$	1,099.3	8
Sodium hyposulfite	$Na_2S_2O_4.2H_2O$	1,104.3	9
Sodium sulfoxylate	Na_2SO_2	(?)	
(Hydrogen sulfide)	H_2S	(912.5)	6
Sodium disulfide (pH=6.62)	Na_2S_2	602.3	8
Ferrous sulfide	FeS	166.1	9
Sodium sulfide (pH=6.70)	$Na_2S.9H_2O$	256.4	10
Sodium thiosulfate	$Na_2S_2O_3.5H_2O$	1,029.8	4
Sodium dithionate	$Na_2S_2O_6$	2.4	0
Sulfamic acid	NH_2SO_3H	949.3+	4

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.
[2] Maximum.

Results with sulfide and disulfide require further explanation (see also tables 2 and 5). An old sample of sodium sulfide gave maximum yields, as did also the sodium disulfide formed by solution of the calculated amount of sulfur in a 9-percent solution of the sulfide. Inasmuch as this same sample has been used in a previous study (*16*) and found unserviceable as a source of sulfur, additional tests were made to clear up the discrepancy. The data obtained indicated that the sulfur of old and presumably altered (oxidized) samples of sodium sulfide (or disulfide prepared with it) was fully assimilable. Fresh samples gave poor growth, showing symptoms of sulfur deficiency. The transformation of nonassimilable sulfur of sodium sulfide and disulfide to assimilable sulfur was found to be accelerated even by slight alkalinity. Yields with disulfide were uniformly greater than with sulfide. This difference will be discussed in connection with tables 2 and 5.

Growth with sulfur as hydrogen sulfide was almost optimum, but little weight is placed on these data, inasmuch as passage of gas through the nutrient solution (5 minutes) caused precipitation of much sulfur. As will be noticed, ferrous sulfide served poorly as a source of sulfur, though decomposed by the organism, unless a large excess was present. Turbidity of the substrate in the latter case would indicate that a similar decomposition of hydrogen sulfide presumably occurred as with passage of the gas.

An oxidation product of sulfuric acid, namely, persulfate, proved freely available as a source of sulfur, and it is believed that it would have given maximum yields with adjustment of other components of the nutrient solution. A like interpretation of the results with sulfamic acid is also probable. Only two of the six known thionic acids could be tried as sources of sulfur, namely, thiosulfate and dithionate. Sodium thiosulfate gave maximum yields when used as a source of sulfur. Practically no growth occurred with sodium dithionate.

TABLE 2.—*Effect of acidity and age of sample on the utilization of sulfide and disulfide by Aspergillus niger (4 days' growth at 35° C.)*

Source of sulfur	Initial acidity of nutrient solution	Yield per 2.5 gm. of sucrose	Starch [1] in mycelium	Sporulation [2]
	pH	*Mg.*		
Sodium sulfide (old sample)	6.62	1,088.6	1	6
	7.83	1,140.4	0	9
Sodium sulfide (new sample)	6.62	254.6	0	10
	7.69	446.3	0	10
Sodium disulfide (old sample)	6.70	1,086.9	2	5
	7.46	1,111.9	0	9
Sodium disulfide (new sample)	6.70	602.3	4	8
	7.61	986.1	1	6
Sodium sulfate (control)	5.78	1,188.9	0	6
	7.08	1,173.0	0	8

[1] Starch is rated from 0 (none) to 5 (very profuse).
[2] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.

Further insight into the mechanism of sulfide and disulfide utilization is afforded by the data of table 2. Attention is called to the increased yields accompanying increased age of the sample of sulfide. A slight initial alkalinity of the nutrient solution led in all cases to an increase in yield, particularly with the fresh and presumably unoxi-

dized sample. This is interpreted to imply that sulfide and disulfide are more rapidly altered in alkaline solution to forms assimilable by *Aspergillus niger*. It is a matter of general knowledge to mycologists that even higher acidities than are here dealt with promote increased growth of this fungus.

Insufficient evidence is available to form a final opinion on the cause of the higher yields with disulfide as compared with sulfide. The use of sulfur washed with alcohol and water for the preparation of disulfide has little effect on yield. Sulfur is quite difficult to obtain pure and free from oxygen except by recrystallization in carbon disulfide and subsequent distillation in nitrogen (*1*). Washing the sulfur as aforesaid, prior to the preparation of disulfide, would appear of little value in this connection (*8*).

The possibility exists that chemical transformations that result in the formation of assimilable sulfur are more readily undergone by disulfide than by sulfide. Stock solutions of the former alter more rapidly on standing than do those of the sulfide (yield of fungus).

ASSIMILABILITY OF SULFUR IN CYSTINE, METHIONINE, AND THIAMIN [4]

Cysteine and oxidized derivatives (table 3) gave maximum growth when supplied as sources of sulfur. The same is true for taurine, which might be considered as formed from cysteic acid by decarboxylation, and for taurine disulfoxide. Deaminization of taurine to form ethane sulfonic acid does not diminish the assimilability of the contained sulfur, as is shown by the yields obtained with potassium ethane sulfonate. Benzylation of the sulfhydryl group of cysteine was only moderately effective in decreasing the availability of cysteine sulfur. Methionine, cystine, cysteine, and homocystine were equally effective as sources of sulfur supply. A test with thiamin chloride revealed that this essential metabolite in the nutrition of the fungi was unable to serve as a general source of sulfur supply.

TABLE 3.—*Growth data for Aspergillus niger after 4 days at 35° C. with metabolites or their derivatives providing 25 mg. of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. of sucrose	Sporulation [1]
		Mg.	
No sulfur (control)		19.4	2
Cysteic acid	$CH_2(SO_3H).CH(NH_2)COOH$	1,140.7	8
Cysteine sulfinic acid	$CH_2(SO_2H).CH(NH_2)COOH$	1,239.0	8
Cysteine disulfoxide	$(C_3H_6O_2N)SO_2.S(C_3H_6O_2N)$	1,161.5	8
l-Cystine	$[-S-CH_2CH(NH_2)COOH]_2$	1,218.9	8
Cysteine hydrochloride	$HSCH_2CH(NH_2.HCl).COOH$	1,087.3	8
Taurine	$NH_2CH_2CH_2SO_3H$	1,039.5	8
Taurine disulfoxide	$NH_2CH_2CH_2SO_2.SCH_2CH_2NH_2$	1,126.9	8
Potassium ethane sulfonate	$C_2H_5SO_3K$	1,176.9	8
dl-Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$	1,183.2	8
Homocystine	$[-SCH_2CH_2CH(NH_2)COOH]_2$	1,103.8	4
S-benzylcysteine	$C_6H_5CH_2.SCH_2CH(NH_2)COOH$	623.3	8
Thiamin chloride [2]	$C_{12}H_{17}N_4SOCl$	80.5	4

[1] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores were black.
[2] Only 22.2 mg. S/L.

[4] Lanthionine $[(HO_2C.CH(NH_2)CH_2)_2S]$ and "optically inactive cystine" were obtained, subsequent to completion of this manuscript, from Dr. D. B. Jones and Dr. M. J. Horn, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture. The yield with this new amino acid was only 159.3 mg., while that with "optically inactive cystine" was 1,099.2 mg., or about that obtained with *l*-cystine.

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The state of oxidation of sulfur in anabolites is immaterial, therefore, in their use by the organism as a general source of sulfur supply. This is in marked contrast to the results previously discussed on the assimilation of inorganic sulfur. It is evident, therefore, that a clear distinction must be made in the nutrition of this fungus between compounds requiring digestion and those utilized without a preliminary alteration, because they are identical with substances (anabolites) necessarily formed during development.

ASSIMILABILITY OF SULFUR IN MISCELLANEOUS ORGANIC COMPOUNDS

Growth data for *Aspergillus niger* with sulfur compounds of the mercaptan-sulfonate series, the sulfide-sulfone series, and miscellaneous compounds are given in table 4. The slight increases in yield with *n*-propyl sulfone, *n*-amyl disulfide, benzyl isothiourea, and benzoyl persulfide are perhaps fortuitous and due to impurities of other sulfur compounds. An interesting feature of these responses is the failure of ethyl, propyl, and heptyl mercaptans to serve as sources of sulfur. It can probably be assumed that all alkyl mercaptans are useless as sulfur supply. Nevertheless, ethane sulfonic acid, an oxidation product of ethyl mercaptan, furnishes sulfur in readily available form, as does isoamyl sulfonic acid (table 5).

TABLE 4.—*Growth data for Aspergillus niger after 4 days at 35° C. with miscellaneous organic compounds providing 25 mg. or more of sulfur per liter of solution*

Source of sulfur	Formula	Yield per 2.5 gm. of sucrose	Sporulation [1]
		Mg.	
No sulfur (control)		4.7	0
Dimethyl sulfone	$(CH_3)_2SO_2$	6.7	1
Ethyl mercaptan	C_2H_5SH	3.1	0
n-Propyl mercaptan	C_3H_7SH	6.3	0
n-Propyl sulfide	$(C_3H_7)_2S$	6.8	0
n-Propyl sulfone	$(C_3H_7)_2SO_2$	243.8	8
n-Propyl disulfide	$C_3H_7S.SC_3H_7$	3.7	0
n-Heptyl mercaptan	$C_7H_{15}SH$	5.5	0
n-Heptyl sulfide	$(C_7H_{15})_2S$	6.4	0
n-Amyl disulfide	$(C_5H_{11}S)_2$	241.2	1
Sodium hydroxymethane sulfinate	$HOCH_2SO_2Na$	1,193.8	9
Thioglycolic acid	$HSCH_2COOH$	0	0
Potassium ethyl xanthate	$C_2H_5OCS.SK$	0	0
Benzyl isothiourea hydrochloride	$C_6H_5CH_2SC(NH_2)NH$	152.4	8
Di(β-phenylpropiophenone)-β-sulfide	$[C_6H_5CH(CH_2COC_6H_5)]_2S$	13.1	1
Thiourea	$(NH_2)_2CS$	7.6	0
Sulfonal	$(CH_3)_2C(SO_2.C_2H_5)_2$	6.2	0
Diphenyl thiocarbazone	$C_6H_5N.NCSNHNHC_6H_5$	23.7	2
Sodium benzene sulfinate	$C_6H_5SO_2Na$	29.5	2
Diphenyl sulfone	$(C_6H_5)_2SO_2$	15.3	2
Diphenyl disulfide	$C_6H_5S.SC_6H_5$	0	0
Diphenyl sulfoxide	$(C_6H_5)_2SO$	16.6	2
Benzoyl persulfide	$(C_6H_5CO.S)_2$	188.5	1
Benzyl disulfide	$(C_6H_5CH_2S)_2$	2.1	0
Benzyl sulfide	$(C_6H_5CH_2)_2S$	0	0
Trithiomethylene	$(HCHS)_3$	2.6	0
Thioacetic acid	$CH_3CO.SH$	0	0
Thioacetamide	$CH_3CS.NH_2$	600.5	2
β,β-Dihydroxyethyl sulfide	$(HOCH_2CH_2)_2S$	460.8	3
Potassium dithiooxalate	$(COSK)_2$	750.5	1

[1] Sporulation is rated from 0 (sterile) to 10 (maximum). All spores were black.

TABLE 5.—*Effect of barium ion on growth of Aspergillus niger (4 days at 35° C.) with various sulfur compounds*

Source of sulfur (25 mg. of sulfur per liter)	Yield per 2.5 gm. of sucrose	
	No barium added	Barium added (120 mg. per liter)
	Mg.	*Mg.*
Sodium sulfate	1,209.7	414.8
Taurine	1,190.4	1,024.1
Cysteic acid	1,147.1	1,131.9
Potassium ethane sulfonate	1,216.4	1,204.3
Sodium iodomethane sulfonate	116.6	83.7
Sodium isoamyl sulfonate	1,183.0	885.7
Sodium 2-bromethane sulfonate	40.0	35.5
Thioacetamide	481.0	433.1
β, β-Dihydroxyethyl sulfide	568.2	475.5
Potassium dithiooxalate	463.2	454.0

Trial of alkyl sulfinates could not be made, but growth with sodium hydroxymethane sulfinate (sodium formaldehyde sulfoxylate) was fully equal to that of any source of sulfur. Its sulfur is in the lowest stage of oxidation compatible with maximum efficiency of assimilation of all organic compounds investigated except methionine, cysteine, homocystine, and cystine. It is important to note that, like all sulfinates, it may be considered a derivative of sulfoxylic acid, whose sulfur is in the lowest stage of oxidation for maximum efficiency of assimilation of all inorganic compounds investigated. That is to say, sulfoxylate and sulfinate are synonymous terms, the one used for inorganic compounds, the other for organic. The chemistry of sodium hydroxymethane sulfinate has been discussed by Bazlen (*3*) and Whitmore (*20*).

The difference in nutritive value of sulfur in sulfhydryl and disulfide anabolites (cysteine, cystine, and methionine) and of catabolites (alkyl mercaptans, alkyl disulfides) is sharp and clean-cut. It would indicate that the important factor, at least in metabolism, is molecular configuration as a whole, and not the configuration of specific groups in the molecule.

Other interesting features are nonassimilability of benzene sulfur derivatives, the availability of sulfur in dithiooxalic acid in contrast to the unavailability of that in thioacetic acid, the effect of an amino group attached to the same carbon to which sulfur is attached by a double bond (thioacetamide), and the effect of introduction of oxygen into the alkyl groups of ethyl sulfide (β, β-dihydroxyethyl sulfide). These citations furnish examples of the influence of adjacent groups in the organic molecule upon the rate and ease of assimilation of its contained sulfur.

Availability of a number of alkyl sulfonates permitted a closer experimental study of the method whereby they are utilized as a source of sulfur by the fungus. Several possibilities exist. Sulfur may be used only after hydrolytic removal from the molecule as free sulfonic, sulfinic, or sulfenic acid, depending on concomitant reduction. The nature of the alkyl group would be of little influence in this event. If the barium ion is without effect upon sulfur utilization, it can be assumed that its hydrolytic removal does not occur as the sulfate (*16*).

If the composition of the alkyl residue does determine the extent of sulfur assimilation, the possibility exists that sulfur is assimilated only in combination with a specific carbon group, or that the presence of substituents may prevent nonhydrolytic removal of sulfur.

Examination of table 5 discloses that a considerable difference in composition of the alkyl group [CH_3CH_2— as compared with $(CH_3)_2CH_2CH_2CH_2$—] had no influence on the efficiency of sulfur utilization. However, the presence of halogen substituents in sodium iodomethane sulfonate and sodium 2-bromoethane sulfonate definitely prevented the assimilation of sulfur. It is improbable that toxicity plays a part in this response, inasmuch as the former compound is claimed to be inert in the animal after ingestion and suitable for X-ray diagnoses. The possibility is slight that the presence of halogen prevents the hydrolytic removal of sulfur, since in compounds such as trichloracetic acid and trichloracetaldehyde its effect is to weaken the carbon-to-carbon bond. It seems likely, therefore, that cleavage of sulfur from these compounds by the organism is nonhydrolytic, a residue with a double bond being formed.

Several reasons render probable the interpretation that the sulfur is split off in the form of free sulfoxylic acid (HSO_2H). The ineffectiveness of the presence of barium ion would indicate that sulfur is not removed hydrolytically or otherwise as sulfuric acid. Its removal as free sulfurous acid (HSO_3H) is possible, of course, but improbable from the chemical point of view. Lastly, alkyl sulfinate is equivalent to sulfate-sulfur in assimilability and may undergo this type of cleavage with production of free sulfinic acid (HSO_2H). Whether, however, sulfur is reduced still further before cleavage to free sulfenic acid (HSOH) must await further investigation. As far as the evidence goes, the indications are that sulfur is removed from organic compounds as free sulfinic acid, and that the process is probably enzymatic and due to the presence of "desulfinase."

Since the splitting off of the sulfonic acid group is doubtful under these conditions from what is known of its chemistry, the evidence would indicate that reaction occurs only after reduction at least to sulfinate has occurred. This conclusion is identical with that arrived at on the basis of the data on assimilation of inorganic sulfur and on that of sodium hydroxymethane sulfinate.

EFFECTS OF TRACE-ELEMENT DEFICIENCIES WITH VARIOUS SULFUR COMPOUNDS

Further data on the relation of inorganic sulfide and disulfide to growth of *Aspergillus* are brought out in table 6. The yields would indicate that age of sample and alkalinity of the nutrient solution favor the rapid transformation of sulfide and disulfide into forms readily assimilable by the fungus. The addition of sodium disulfide to the nutrient solution invariably causes the formation of a profuse precipitate, presumably of finely divided sulfur. This precipitate largely disappeared during the course of growth of the fungus. A precipitate is also formed with sodium thiosulfate in acid solution and disappears similarly.

TABLE 6.—*Effect of age of sulfide and disulfide sulfur on growth of Aspergillus niger for 4 days at 35° C. and on the responses of the fungus to deficiencies in trace elements*

Element omitted	Sodium sulfide (old sample)				Sodium disulfide (old sample)				Sodium sulfide (fresh sample)				Sodium disulfide (fresh sample)				Sodium sulfide (fresh sample in non-alkaline solution)			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			
None	1,140.4	100.00	1.83	0	9	1,111.9	100.00	1.86	0	9	446.3	100.00	2.01	0	10	986.1	100.00	1.88	1	6	359.0	100.00	2.14	0	10
Fe	161.2	14.14	2.00	0	4	172.4	15.51	1.99	0	4	102.1	22.88	2.23	0	4	88.5	8.98	2.26	0	2	232.7	64.82	1.99	0	10
Zn	416.7	36.54	1.79	0	10	414.6	37.29	1.81	0	10	310.1	69.46	2.02	0	10	242.5	24.59	1.96	0	10	280.2	80.82	2.14	0	10
Cu	1,154.9	101.27	1.93	0	6, br	1,144.9	102.97	2.05	0	6, t	464.4	104.05	1.85	0	8	892.2	90.48	2.07	1	2	293.4	81.71	2.04	0	10
Mn	651.7	57.15	1.49	3*	0	639.3	57.50	1.50	3	0	369.5	82.79	1.64	3*	2	456.3	46.27	1.64	3*	1	236.8	65.96	1.67	2	0
Mo	1,126.4	98.77	1.78	0	9	1,065.3	95.81	1.83	0	4	532.6	114.34	1.84	1	10	1,042.7	105.74	1.95	0	6	322.9	90.21	2.02	0	10
Ga	1,128.6	98.96	1.85	0	9	1,111.8	99.99	1.84	0	8	501.2	112.19	1.91	1	10	979.2	99.30	1.97	0	6	315.6	87.89	2.11	0	10
Maximum [3]	1,146.1					1,159.8					539.4					1,102.8					388.1				
C. U. [4]		45.84					46.39					21.58					44.11					15.52			
pH [5]			7.83					7.46					7.60					7.61					4.18		

[1] Starch is rated from 0 (none) to 5 (very profuse), an asterisk denoting the immediate formation of a blue color on addition of iodine. Starch in substrate indicated by parentheses. Erythrodextrin indicated by E.

[2] Sporulation is rated as 0 (sterile) to 10 (maximum). All spores are black unless otherwise stated; br = brown, t = tan, y = yellow, w = white.

[3] Maximum individual yield.

[4] Coefficient of utilization or yield per 100 gm. of sucrose.

[5] Initial acidity of nutrient solution.

The acidities employed in the acid nutrient solution in table 6 are not considered too high, since Mellor (*8*) states that a solution of 0.5 N-HCl (pH=0.36±) has been found to dissolve 2.54 times its volume of hydrogen sulfide. This is more than ample to supply the quantity of sulfur required for maximum growth. The data for sodium disulfide in acid solution have been omitted, since yields were maximum owing to complete aging of disulfide.

The sample of sulfur used in the preparation of the disulfide gave appreciable increases in growth when used as a sole source of sulfur. It is possible, therefore, that elemental sulfur in a very finely divided form is capable of utilization by the fungus in the presence of reduced inorganic sulfur compounds because it is more readily converted to assimilable sulfur chemically. Trials with equal quantities of elemental sulfur and of sulfur as sulfide, hyposulfite, sulfite, or thiosulfate gave increases over and above those attributable to the individual constituents with sulfur and thiosulfate and with sulfur and hyposulfite.

Results with trace elements proved disappointing in view of the insolubility of sulfides (except gallium). The marked acidification of the substrate during the course of development was probably responsible for the negative results obtained. As previously mentioned, ferrous sulfide readily dissolves under these conditions. Nevertheless, cupric sulfide is insoluble at much higher acidities than were here encountered. The data would indicate, however, that the usual trace elements were required for growth. Deficiency of individual trace elements was without marked effect on acidity of the cultures at harvest, or on starch in the mycelial felts, except in those cases in which addition of manganese was omitted.

The effects of a trace-element deficiency with various sulfur compounds serving as sources of sulfur in the nutrition of *Aspergillus niger* are tabulated in table 7. It is evident that the trace elements required with sulfate sulfur are also needed for growth with other inorganic sources of sulfur, no matter in what stage of reduction. A similar condition was found to exist with cystine, taurine, methionine, and their derivatives. No evidence was obtained that reduction of inorganic sulfur to a form suitable for conversion into organic sulfur is specifically associated with the presence of one of the essential trace elements. It should be realized, on the other hand, that further refinement of experimental technique may lead to quite different results in the future. Results on sporulation, formation of starch, and acidity of the substrate at harvest were also much the same with all sources of sulfur.

Interpretation of the data with propyl sulfone requires further study, though growth with this compound has been previously assumed to be due to other sulfur impurities. Dimethyl sulfone cannot supply assimilable sulfur. Nor were alkyl sulfoxides available for trial. The fact that propyl sulfide cannot be utilized should not be considered as confirmatory evidence, since ethyl mercaptan cannot be assimilated, whereas its oxidation product, ethyl sulfonate, can be used.

TABLE 7.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C., with various sources of sulfur supply* [1]

Element omitted	Magnesium sulfate					Sodium thiosulfate					Sodium bisulfite					Taurine					Potassium ethane sulfonate					Cysteine hydrochloride				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,041.7	100.00	3.14	3	8	917.5	100.00	2.53	4	4	1,020.8	100.00	2.76	2	8	1,006.5	100.00	2.24	4	8	1,023.6	100.00	2.41	3	8	1,006.1	100.00	2.41	4	8
Fe	71.3	6.61	2.39	0	1	78.0	8.51	2.39	0	2	134.6	13.18	2.08	0	1	193.0	19.17	2.11	1	4	100.2	9.79	2.19	0	1	141.2	14.03	2.24	1	4
Zn	134.9	12.96	2.33	0	1	30.8	3.36	2.99	0	1	47.9	4.69	2.59	(?)	1	22.3	2.22	2.96	0	1	52.8	5.16	2.57	(?)	1	41.1	4.08	2.75	1	1
Cu	930.9	89.40	3.14	4	2	650.7	70.93	2.30	5*	1	742.0	72.69	2.57	3	1	873.2	86.76	2.25	4	3	817.7	79.89	2.47	3**	1	954.8	84.94	2.47	2	2
Mn	976.5	93.94	1.98	4	2	812.5	88.56	2.07	2	1	1,069.3	107.69	1.98	4	1	994.1	98.77	2.02	4	1	1,100.4	107.50	2.05	4	2	1,087.3	108.07	2.08	5	2
Mo	763.0	73.24	2.33	4	3	825.7	90.00	2.39	3	4	813.5	79.69	2.12	5*	2	876.5	87.09	2.09	4	4	981.7	95.96	2.36	4	6	950.8	94.57	2.19	4	6
Ga	972.3	93.33	3.09	3	6	823.3	89.74	2.39	5*	2	979.1	95.91	2.82	3	6	926.4	92.04	2.13	4	4	964.1	96.14	2.51	3	6	973.9	96.87	2.31	4	7
Maximum	1,080.0					1,029.8					1,099.3					1,039.5					1,100.4					1,087.3				
C. U		43.20					41.19					43.97					41.58					44.02					43.49				
pH			7.01					7.28					7.12					6.94					7.00					6.40			

[1] See footnotes to table 6.

TABLE 7.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C., with various sources of sulfur supply*—Continued

Element omitted	*dl*-Methionine					*n*-Propyl sulfone					Cysteine disulfoxide					Cysteic acid					Sodium hyposulfite					Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	932.7	100.00	2.16		3	243.8	100.00	2.19		0	806.2	100.00	2.25		5	1,020.8	100.00	1.86	0	8	1,082.6	100.00	3.10		2	1,183.3	100.00	1.83	1	8
Fe	124.5	13.34	2.10		0	156.4	64.17	2.26		0	53.4	6.62	2.48		0	267.3	26.19	1.94	0	6	116.0	10.71	2.13		0	343.8	29.06	1.74	2	8
Zn	41.3	4.43	2.75		4	103.4	42.42	5.77		0	189.0	23.45	2.09		4	26.6	2.61	2.79	0	2	129.1	11.92	2.52		0	486.7	41.14	1.74	0	10
Cu	840.8	90.15	2.21	5 "E"	2,y	217.6	89.26	2.14	0 (1)	8	681.8	84.57	2.39	4 (1)	0	1,069.6	104.78	2.21	0	4,t	813.1	75.15	2.71		3	1,176.1	99.42	1.87	1	4
Mn	912.1	97.79	1.96		4	243.1	99.73	2.11	2 (1)	10	1,082.3	134.24	2.13	5 (2)	0	576.5	56.48	1.62	0	4	1,059.1	97.82	1.85		3	1,044.2	88.26	1.62	1	4
Mo	702.0	75.26	1.90	5*	4	271.5	111.37	1.95	2 (1)	10	579.9	71.93	1.85	5*(5)	0	1,012.5	99.19	1.86	0	6	699.3	64.60	1.91	5*(5)	3	1,192.0	101.26	1.84	0	6
Ga	876.6	93.98	2.11	4	6	186.3	76.44	2.25	1	6	772.3	95.79	2.22	5	1	1,107.7	108.52	1.94	0	8	1,029.2	95.06	3.01	2	8	1,044.2	88.26	1.62	1	4
Maximum	963.0					297.3					1,082.3					1,140.7					1,104.3					1,200.7				
C. U		38.52					11.89					43.27					45.63						44.17					48.03			
pH				6.89				7.09					6.84					6.62					6.88					7.39		

EFFECTS OF NUTRIENT-SOLUTION PURIFICATION WITH VARIOUS SULFUR SOURCES

Results with trace-element deficiencies after nutrient-solution purification with calcium carbonate (table 8) emphasize the results obtained with unpurified solutions. There would seem little doubt that all the essential trace elements are required irrespective of the source of sulfur. Results with iron and zinc deficiencies were good from the experimental point of view with every sulfur compound tested. Omission of copper led to the greatest decrease in growth with sodium hydroxymethane sulfinate. Best results on manganese were obtained with methionine. Cysteic acid gave maximum decreases in yield on omission of molybdenum and of gallium, but the results were poor. Little weight is placed on the data for *n*-propyl sulfone. Interpretation of these results, however, must await further evidence that they are not fortuitous.

Increased mycelial deposition of starch occurred most frequently with a deficiency in manganese, sometimes with a deficiency in molybdenum, and more rarely with a deficiency in copper or iron. This phenomenon was usually accompanied by specially high increases in acidity of the substrate and a sharp diminution in sporulation. In no instance did similar responses accompany the omission of zinc. Since yields on omission of zinc were far less than on omission of copper, manganese, or molybdenum, these differences may be only concentration effects and not specific.

DISCUSSION OF RESULTS

The growth data on sulfur utilization with compounds of the sulfide-sulfate series and the mercaptan-sulfonate series would appear to justify the assumption that reduction is the normal preliminary process in the utilization of sulfur compounds. This has been assumed in the case of sulfate assimilation (*2*, *4*, *12*). These data would indicate, however, that reduction may be carried too far. When carried beyond a certain stage (sulfoxylate or sulfinate) the nutritive value of sulfur compounds disappears. Evidently the reverse process of oxidation of sulfur is performed by the fungus with great difficulty, if at all.

A distinction should be made in this connection as concerns metabolites since the organism was able to assimilate all cystine derivatives regardless of the state of oxidation of the contained sulfur. Metabolites, or their derivatives, would appear to follow a definite metabolic channel whether synthesized by the fungus or added to the solution as a nutrient. It is possible that all assimilated sulfur passes through the amino acid stage, since cystine and methionine afford excellent sources of sulfur for general use in metabolism, equal to that of sulfate-sulfur or any other. However, it is not definitely known whether cystine and methionine are assimilated as a whole when fed to the fungus, though this is assumed to be the case. Thiamin chloride was practically unavailable as a general source of sulfur. The fungus does not suffer from the inability of mammalian forms to convert cystine into methionine.

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate* [1]

Element omitted	Magnesium sulfate					Sodium thiosulfate					Sodium bisulfite					Taurine					Potassium ethane sulfonate					Cysteine hydrochloride				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,212.6	100.00	2.54	0	10	1,355.5	100.00	2.26	0	9	1,272.5	100.00	1.97	0	8	832.5	100.00	1.91	2	8	1,252.9	100.00	2.09	0	8	1,305.3	100.00	2.88	0	10
Fe	3.2	.26	3.77	0	0	6.0	.44	3.27	0	1	3.2	.25	3.28	0	0	0	0	6.25	0	0	6.1	.48	3.57	0	0	18.0	1.38	2.85	0	1
Zn	87.8	7.24	2.67	0	6	15.6	1.15	3.00	0	1	21.0	1.65	2.88	0	2	24.5	2.94	3.01	0	1	17.5	1.40	2.89	0	1	33.8	2.59	2.83	0	2
Cu	922.5	76.08	2.34	2	2,y	1,169.3	83.99	2.21	0	0	1,120.3	88.04	2.19	0	4,y	362.3	43.52	1.77	1	4,t	595.4	47.52	1.72	2 (2)	1,t	858.4	65.76	2.28	0	0
Mn	573.0	47.25	1.58	5* (4)	0	430.1	31.71	1.59	4* (2)	0	482.9	37.95	1.56	5* (5)	0	386.2	46.39	1.55	5* (2)	0	441.3	35.22	1.57	5* (3)	0	539.1	41.30	1.58	5* (5)	0
Mo	1,174.3	96.84	2.29	0	4	1,222.6	90.13	1.93	0	6	1,136.8	89.33	1.69	0 (2)	6	675.6	81.15	1.61	5 (5)	6	1,180.4	94.21	1.98	0	4	1,172.2	89.80	1.91	4	4
Ga	1,266.0	104.41	2.95	0	10	1,332.3	98.20	2.40	0	9	1,245.2	97.85	2.08	0	8	746.6	89.68	1.85	2	7	1,271.6	101.49	2.25	0	6	1,336.6	102.40	2.74	0	8
Maximum	1,266.0					1,362.0					1,300.2					887.9					1,327.7					1,338.2				
C. U.		50.64					54.48					52.00					35.52					53.11					53.53			
pH			7.19					7.09					7.06					7.24					7.41					7.07		

[1] See footnotes to table 6.

TABLE 8.—*Effects of trace-element deficiencies on growth of Aspergillus niger for 4 days at 35° C. with various sources of sulfur supply after purification of the nutrient solution with calcium carbonate*—Continued

Element omitted	*dl*-Methionine					*n*-Propyl sulfone					Cysteine disulfoxide					Cysteic acid					Sodium hyposulfite					Sodium formaldehyde sulfoxylate (sodium hydroxymethane sulfinate)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	1,009.0	100.00	1.89	0	6	54.9	100.00	3.01	0	4	1,229.9	100.00	2.09	0	6	672.9	100.00	1.79	0	8	1,303.5	100.00	2.08	0	8	1,281.7	100.00	2.25	1	6
Fe	0	0	6.37	0	0	0	0	6.61	0	0	3.1	.25	3.48	0	0	3.6	.53	3.32	0	1	5.6	.43	3.09	0	2	18.3	1.42	2.97	0	1
Zn	28.3	2.81	2.87	0	4	15.0	27.25	3.18	0	4	30.4	2.47	2.90	0	3	17.3	2.58	3.02	0	2	16.3	1.25	2.93	0	2	5.7	.45	3.00	0	0
Cu	1,146.0	113.58	2.06	0	4,y	28.9	52.66	3.03	0	4	1,217.5	99.03	3.32	0	3,br	500.9	74.45	1.74	0	8	750.5	57.58	1.78	2	4,br	466.5	36.40	1.67	5* (3)	0
Mn	225.8	22.38	1.78	5*	0	28.3	51.43	2.67	0	4	335.6	27.37	1.62	5* (2)	0	261.1	38.81	1.68	5*(0)	0	373.7	28.66	1.55	5*(3)	0	523.9	40.88	1.53	5* (3)	0
Mo	826.7	81.94	1.60	5* (5)	4	44.1	80.33	2.91	0	4	977.2	87.24	1.76	0 "E"	4	544.8	80.99	1.61	0 (2)	8	1,267.0	97.20	1.96	0	6	1,189.7	92.82	1.99	1	4
Ga	1,041.0	103,17	1.95	0	6	38.3	69.67	2.99	0	4	1,259.2	102,38	2.19	0	6	555.2	82.53	1.71	0	8	1,305.5	100.15	2.16	0	8	1,264.6	99.67	2.26	1	6
Maximum	1,146.0					74.2					1,259.2					834.8					1,361.5					1,326.2				
C. U		45.84					2.97					50.37					33.39					54.46						53.05			
pH			7.00					7.23					7.12					7.01					7.08					7.86		

If metabolites serving as nutrients may be pictured as passing into the normal channels present in the organism for their use, the case of nonmetabolites falls into a different category. Nutrients must first be altered to a form suitable for assimilation (digestion) and so brought into normal metabolic channels.

While mercaptans are formed by various organisms, they are probably products of catabolism (waste products) and therefore without a normal channel for anabolism. In this case also, sulfur as alkyl mercaptan was unassimilable, whereas it became readily assimilable after oxidation to alkyl sulfonate. Possibly alkyl sulfinates [5] (RSO_2H) or alkyl sulfinates (RSOH) will be found equally effective. It appears probable, therefore, that with organic compounds also a basic distinction exists in the absence of oxygen and its presence in combination with sulfur, or in an adjacent group. The partial utilization of substances like β, β-dihydroxyethyl sulfide, whereas ethyl sulfide presumably cannot supply sulfur, illustrates the point under discussion.

Though sulfur in alkyl sulfones is combined with oxygen, these proved to be poor sources of sulfur supply. The reason for this may be the inability of the fungus to effect their reduction. It is known that even nascent hydrogen cannot bring about their reduction. Investigation of the assimilability of sulfur in alkyl sulfoxides should help to decide whether this is the correct or sole interpretation.

Other factors of molecular configuration may prevent the utilization of organic sulfur. Sulfur attached to the benzene ring was unsuitable for use in assimilation irrespective of its state of oxidation. However, further tests may reveal that the presence of adjacent groups may considerably modify this response. Sulfur in the thiazole ring (thiamin chloride), or other rings presumably, will probably prove of little value as a nutrient, unless special conditions exist that permit of its ready release in combination with oxygen. The fungus seems unable to avail itself of sulfur attached to carbon, as in thiourea, though again exceptions may exist. The number of sulfur derivatives available for test was far too small to arrive at final conclusions. There is some indication that the presence of adjacent groups (HO—, CO═, NH_2—) may increase the availability of sulfur.

Nevertheless, the different lines of approach employed in the study of sulfur assimilation by *Aspergillus niger* have yielded an identical conclusion. Sulfur, it was found, was reduced and transformed to sulfoxylic acid when supplied as sulfate or alkyl sulfonate. Hydroxymethane sulfinate, a derivative of sulfoxylic acid, was as effective as sulfate in supplying sulfur. Miscellaneous compounds effective as sources of sulfur supply seem to require the presence of oxygen in the molecule. A further reduction to sulfenate before assimilation is a theoretical possibility, though no alkyl sulfenates are known. Whether other available sulfur compounds (β, β-dihydroxyethyl sulfide, etc.) also decompose to form sulfinate is unknown and must await further investigation. It should not be overlooked, however, that anabolites need not conform to this behavior.

Nicolet (*11*) has suggested the possibility of methylene pyruvic acid being a precursor in the utilization by the plant of sulfur in the

[5] A supply of sodium *n*-butane sulfinate ($CH_3CH_2CH_2CH_2SO_2Na$) became available through the kindness of Dr. William H. Ziegler and Dr. Ralph Connor, of the John Harrison Laboratory, University of Pennsylvania, Philadelphia, Pa., after completion of this manuscript. Yield with 25 mg. per liter of sulfur supplied as this compound was 1,173.2 mg. In the presence of barium ion (see table 5) the yield was 768.0 mg.

form of inorganic sulfide or disulfide or as mercaptan, to account for the formation of methionine. Experimental results with *Aspergillus niger* would indicate, however, that the forms of sulfur postulated by Nicolet are unassimilable by this fungus. Other organisms (bacteria) may be capable of assimilating sulfide, disulfide, and mercaptan and may possibly follow the Nicolet course of assimilation. A clear distinction should be made by investigators of this question between utilization for energy and as units for essential metabolites. Experimental proof with aerobic forms would be difficult or impossible because of atmospheric oxidation. Conditions with anaerobes would probably be such as to afford adequate proof.

Nonassimilation of sulfur in sulfide, disulfide, and alkyl mercaptan by *Aspergillus* does not necessarily imply that methylene pyruvic acid does not serve with this organism as a precursor of methionine. The available information might readily be interpreted on the basis of side reactions that lead to the locking of sulfur in unavailable form. Addition of sulfoxylic acid at the double bond of methylene pyruvic acid is quite probable chemically. Other biological data would indicate that forms of sulfur (sulfide, thiourea, etc.) unavailable to *Aspergillus niger* are readily assimilated by certain of the bacteria.

SUMMARY

Alterations in the source of sulfur supply were practically without effect on the trace-element requirements of *Aspergillus niger* Van Tiegh. Iron, zinc, copper, manganese, molybdenum, and gallium were apparently required in approximately equal degree, whatever the state of oxidation of sulfur supplied as a nutrient. The slightly better results obtained through omission of copper with sodium hydroxymethane sulfinate, of manganese with methionine, and of molybdenum and gallium with cysteic acid may prove to have been due to chance.

A survey of the assimilability of inorganic sulfur compounds indicated that sulfur is reduced to sulfoxylate prior to its conversion to organic sulfur. Sulfide and disulfide were not assimilated.

Assimilability of organic sulfur varied with molecular configuration and was also correlated with the presence of attached or adjacent oxygen in the molecule. Alkyl mercaptans, sulfides, and disulfides could not be used as a source of sulfur, whereas alkyl sulfonate and alkyl sulfinate were readily available. Utilization of sulfur in alkyl sulfonates and alkyl sulfinates was considered to depend on their decomposition into free sulfinic acid and an unsaturated residue.

Anabolites, particularly cystine and its derivatives, homocystine, and methionine, were readily available as sole sources of sulfur supply, irrespective of the state of oxidation of their contained sulfur, and were assumed to follow the normal channel for their metabolism. Catabolites and miscellaneous synthetic organic sulfur compounds were assumed to require a process of digestion before assimilation.

ADDENDUM

The data in table 9 were obtained after completion of the manuscript of this paper and are included as a demonstration of the assimilability of alkyl sulfinate by *Aspergillus*. The difficulty with which

compounds of this type can be obtained also made it desirable to report on its utilization as fully as possible.

TABLE 9.—*Growth of Aspergillus niger at 35° C. for 4 days with sulfur supplied as sodium n-butane sulfinate* [1]

Element omitted	Unpurified (25 mg. sulfur per liter)					Purified with $CaCO_3$ (50 mg. sulfur per liter)				
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Starch in mycelium	Sporulation
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	1,041.0	100.00	1.71	0	8	1,262.5	100.00	2.54	0	10
Fe	221.6	21.28	1.96	0	6	6.8	.54	2.94	0	0
Zn	379.6	36.47	1.75	0	10	8.4	.66	2.94	0	0
Cu	1,150.6	110.53	1.84	2	8	1,016.7	80.55	2.05	2	4
Mn	812.0	78.01	1.57	3	4	728.2	57.60	1.53	4	4
Mo	1,025.4	98.50	1.62	1	6	1,096.5	86.85	1.89	2	6
Ga	1,108.7	106.51	1.66	1	8	1,269.0	100.51	2.64	2	10
Maximum	1,150.6					1,295.7				
C. U		46.02					51.83			
pH			7.35					7.47		

[1] See footnotes to table 6.

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